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EXPERIMENTS TO DETERMINE WHETHER THE ALLERGICALLY ACTIVE SUBSTANCE IN RAGWEED POLLEN EXTRACT IS A SINGLE ENTITY OR MULTIPLE*

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DURING the last decade numerous papers have appeared dealing with the nature of the allergically active constituent in pollen extracts and with its standardization. It is not our intention to review this literature in the present communication; suffice it to say, there is a wide divergence of opinion expressed on these subjects. Further elucidation of these fundamental points was a task which we considered warranted further investigation. As indicated in the title, an attempt has been made to determine whether a pollen has a single excitant (or atopen) or several, and in addition whether there is one antibody (or reagin) or several in the serum of individuals sensitive to that particular pollen.

We realized the difficulty, if not the impossibility, of attempting at present to isolate in a state of chemical purity substances possessing allergic activity from pollen extracts. Rather we decided to carry out on a pollen extract a number of fractionating procedures of the mildest character. We have made no attempt, therefore, to isolate pure substances; indeed our fractionation is a very crude one from the chemical point of view; but, on the other hand, we feel that we have avoided, as far as possible, in our treatments, alteration of the chemical identity of any of the original constituents of the pollen extract.

As described in some detail below, pollen† of low ragweed (*Ambrosia artemisiaefolia*) has been extracted by a 5 per cent solution of sodium chloride in water. Some of this extract has been dialyzed and the non-dialyzable portion preserved; some of the extract has been treated with

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†We are again indebted to Knapp and Knapp for providing suitable pollen in the large quantities which were necessary for these investigations.

an equal volume of saturated ammonium sulphate solution, the resulting precipitate and residual solution being later freed from ammonium sulphate and both preserved; some of the original extract has been treated with adsorbent charcoal, and the resulting solution, and a solution of the eluted material from the charcoal preserved. By these simple means we have effected our crude fractionation of the pollen extract. These fractions or extracts are designated in the text of this paper by letters of the alphabet, in the order in which they have thus been outlined by O, D, P.D., S.D., C.A., and C.B.

It was decided to use these alphabetically labeled extracts as scratch test material upon a series of unselected ragweed sensitive individuals, first to determine to what extent the intensities of the resulting reactions to the different fractions varied in the same individual, and second, to observe whether the relative intensities of each fraction varied considerably in different individuals. Following these experiments it was decided to test these extracts by means of local passive transfer (Prausnitz-Küstner reaction) and to note the extent of correlation between the two methods. The protocols, however, were not designed to show quantitative correlation, because this was not considered essential for solution of the problems under consideration. Later it was planned to determine the extent of neutralization in the sensitized sites (obtained by local passive transfer), which the injection of each fraction would induce. This could be inferred by the degree of reaction which would follow a subsequent injection of whole extract. Previous experiments with the serums used in these experiments had shown that the injection of a suitable amount of whole extract* was sufficient to "neutralize" or exhaust completely the sensitized site as judged by the negative result following a reinjection of this whole extract or any other extract or fraction.

All fractions or extracts in the dilutions as noted were employed in scratch tests carried out on a total of forty-two clinical cases of ragweed hay fever. As might be expected, certain of the fractions usually caused little or no reaction, but in a few individuals they did cause as large a reaction as was induced by those fractions usually inducing the maximum results. However, no fraction, or extract used as a control, invariably induced the maximum reaction. Duplicated tests upon some of the subjects gave very satisfactory agreement; in others this was not evident but could be regarded as influenced by the seasonal interval of time between the two tests and the effect of therapeutic injection. Such experiences have been recorded in the literature and are to be expected with any series of repeated tests upon any considerable number of patients. Tabulation of these results gives a very cumbersome chart; we are therefore presenting in Table I the data obtained with five patients

*This consisted of a 5 per cent aqueous ragweed pollen extract: for details see the section dealing with methods of preparation.

whose serums were drawn for the Prausnitz-Küstner tests. These particular individuals were selected because the results afforded by the scratch test showed definite variations one from the other. Serums drawn from such subjects would, we felt, be more likely to provide results which would help to determine unity or not of the allergically active substance in pollen solutions.

TABLE I

RESULTS OF SCRATCH TESTS ON FIVE, SELECTED FROM THE GROUP OF RAGWEED POSITIVE PATIENTS

PATIENT	BA	NU	MC L	MA	DU
EXTRACTS DILUTION	1:100	1:100	1:100	1:100	1:100
734 O	−	−	−	−	+
734 D	++	?	+	+	++
734 S.D.	−	−	−	−	+
734 P.D.	?	−	−	+	+
734 C.A.	+	−	−	+	++
734 C.B.	−	+	−	−	−
334 Whole extract[1]	++	+	−	−	+
434 Sal.[2]	?	+	+	+	++
g & s[3]	++	+	+	−	++
Cho (dry)	−	−	+	−	+
Control	−	−	−	−	−
Wheal in mm.	7				6

[1]Five per cent aqueous extract.
[2]A 5 per cent solution in 5 per cent NaCl.
[3]A 5 per cent solution in Piness fluid, routine treatment set.
To obtain the *exact* dilution based upon weight per volume, the dilution figures should be multiplied by 20. This applies to all dilutions in the paper. The details are given in the section on methods of preparation.

Results Obtained by the Prausnitz-Küstner Test in the First Type of Experiment.—Blood was drawn from each of these five patients and used for local passive transfer as described below.

Ten or more arm sites were prepared on tested and suitable recipients by injecting intracutaneously 0.05 c.c. of the sensitizing serum. Twenty-four to forty-eight hours later each site was reinjected with a different fraction; control injections were made into unprepared sites with as many of the fractions as possible as well as with saline solution. In addition, at least one of the solutions was injected into a normal serum control site. The resulting reactions were recorded and with one serum photographed. Twenty-four to forty-eight hours later each site was reinjected with whole extract.

The reactions induced by this injection varied in reverse proportions to those obtained by the first injection of the different fractions. The intensity of the second reaction (due to whole extract) indicated to what extent there remained unsatisfied reagins in the sensitized sites.

The original scratch tests on the patients were not carried out in a manner which would indicate the greatest dilution in which a minimal reaction was elicited. It is, therefore, not possible to compare exactly the results of the two methods with regard to each fraction in each case. This statement, however, can be made: in no instance did any fraction fail to give at least a small reaction by means of the Prausnitz-Küstner test, though such fraction may have given a negative result with the

same dilution by means of the scratch test. This is illustrated by the comparison of the results recorded in Table I and those in the tables showing the results obtained with the different Prausnitz-Küstner tests.

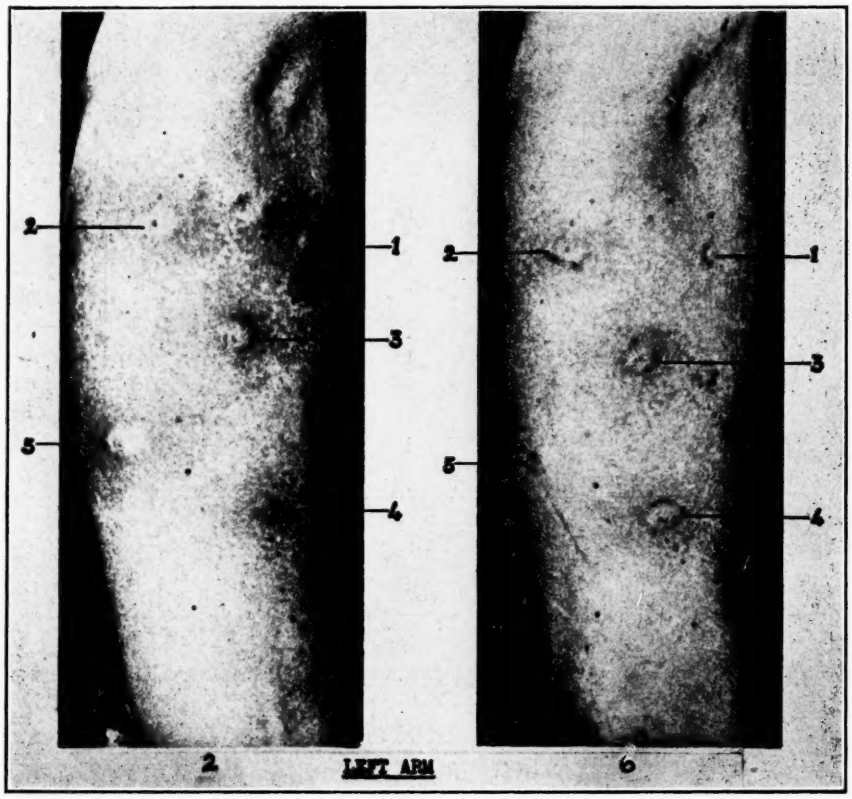

Fig. 1.

TABLE II

(Ba.-Cr., Series 2 and 6) Left Arm

Jan. 10, 1935. Sites 1 to 5 received 0.05 c.c. sensitizing serum (Ba.).
Jan. 12, 1935. Received fractions as follows in 0.05 c.c., with results as recorded.

SITE	FRACTION	RESULT	
1	O	3 plus	
2	D	4 plus	Photograph 2
3	S.D.	3 plus	Fig. 1
4	P.D.	2 plus	
5	Whole extract (134)	4 plus	

Jan. 14, 1935. Received whole extract (334) in all sites.

SITE	RESULT	
1	Site missed	
2	1½ plus	Photograph 6
3	3½ plus	Fig. 1
4	3½ plus	
5	Negative	

All fractions were used in a dilution of 1:100.*

*Methods of making the dilutions are described in the section on methods of preparation of extracts.

Photographs* were made of the allergic reactions with one of the serums in this type of experiment, and we have decided for the sake of

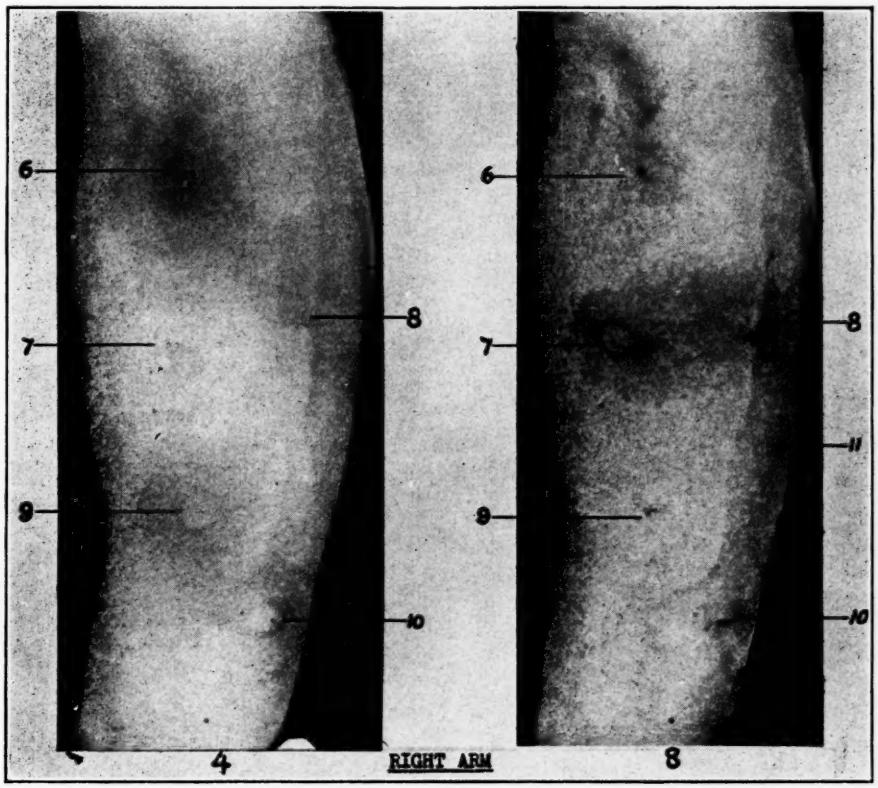

Fig. 2.

TABLE III

(Ba.-Cr., Series 4 and 8) Right Arm

Jan. 10, 1935. Sites 6 to 10 received 0.05 c.c. sensitizing serum (Ba.).
Jan. 12, 1935. Received fractions as follows in 0.05 c.c. with results as recorded.

SITE	FRACTION	RESULT	
6	C.A.	2 plus	Photograph 4 Fig. 2
7	C.B.	1 plus	
8	Saline	Negative	
9	Whole extract (334)	4 plus	
10	Whole extract (1134)	4 plus	

Jan. 14, 1935. All prepared sites and unprepared site 11 received whole extract 334.

SITE	RESULT	
6	1½ to 2 plus	Photograph 8 Fig. 2
7	4 plus	
8	4 plus	
9	Negative	
10	Negative	
11	Negative	

All fractions were used in a dilution of 1:100.

*For the successful development of photographing these skin reactions we are entirely indebted to Dr. J. Craigie of the Department of Epidemiology and Biometrics, Connaught Laboratories, University of Toronto. We take this opportunity of again thanking him for his valuable assistance in this work.

simplicity to use this set (Ba.-Cr.*) which depicts results that are representative of those obtained with the other four serums.

Figs. 1 and 2 show the resulting reactions with one of the five serums selected and five of the fractions, and two whole extracts, only one of which was used for the second reinjection.

Table II records the preparation of sites 1 to 5 on the left forearm. The results which were obtained by injecting these sites with the various fractions forty-eight hours later are given. This table also shows the reactions obtained by reinjecting these five sites with whole extract forty-eight hours later. Photographic reproductions are shown in Fig. 1. Table III records the results for the right forearm with the remaining fractions. The results are shown in Fig. 2. The remaining controls of the fractions into normal sites and a control serum site were not made in this experiment.

The information afforded (as illustrated in the Ba.-Cr. series) by this type of experiment with each of the five selected serums was necessary in order to estimate a suitable dilution of the various fractions which we wished to use in our next type of experiments. Furthermore, since this second type of experiment yields results which bear more directly upon our discussion and conclusion, we shall devote far more attention to them in this paper.

Results Obtained With the Prausnitz-Küstner Test in the Second Type of Experiment.—This consisted in preparing ten or more sites with sensitizing serum. Twenty-four to forty-eight hours later each fraction was injected into two sites. The reactions were photographed after a thirty- to forty-minute interval and also were recorded in plus signs of reaction. In these experiments the backs of the recipients were chosen as affording a greater and more level surface. A third reinjection was made twenty-four to forty-eight hours later by reinjecting one site of each pair with the same fraction and the corresponding site of each pair with whole extract. The reactions were again photographed and recorded as before. A fourth reinjection with whole extract twenty-four to forty-eight hours later was made to show whether any antibody remained in any of the sites. Controls of injecting the various fractions and saline solution into unprepared sites and a site prepared with normal serum were made as indicated in Figs. 3 and 4 and Tables IV, V, and VI.

The results with three different serums are given in support of our conclusions.

In Table IV (Ba.-Cr. series) the primary injections of sites 1 to 10 with sensitizing serum (the same used in Tables I and II) and site 11 with normal serum are given. The reactions which were obtained by in-

*The different series are labeled in this manner to indicate both donor and recipient in the Prausnitz-Küstner reactions. The donor in this report is the ragweed positive individual from whom serum was drawn.

jecting the different fractions forty-eight hours later in paired sites (*1 and 2, 3 and 4,* etc.) are recorded in plus signs. Site 9 shows the maximum reaction resulting from the injection of whole extract; site 10, the negative result with saline solution; and site 11, the normal serum

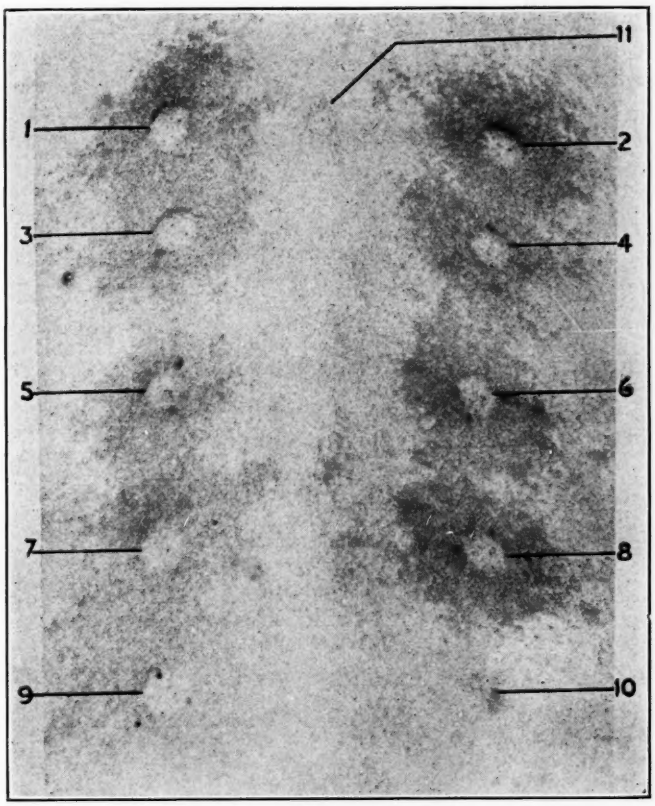

Fig. 3.

TABLE IV

(Ba.-Cr., Series: 1)

March 14, 1935. Prepared sites 1 to 10 received 0.05 c.c. sensitizing serum. (Ba.) Prepared site 11 received 0.05 c.c. normal serum.

March 16, 1935. Prepared sites 1 to 11 received 0.05 c.c. pollen fractions and normal saline as noted with results as recorded.

LEFT			RIGHT		
SITE	FRACTION	RESULT	SITE	FRACTION	RESULT
1	D.	3 plus	2	D.	3 plus
3	S.D.	2 plus	4	S.D.	2 plus
5	P.D.	2½ plus	6	P.D.	2½ plus
7	C.A.	3 plus	8	C.A.	3 plus
9	Whole extract	4 plus	10	Saline	Negative
11	D.	Negative	(normal serum control)		

All fractions were used in a dilution of 1:100.

control, with one of the fractions. Fig. 3 illustrates the identical degree of reaction in the paired sites recorded in Table IV for all paired injections as well as the negative character of the controls.

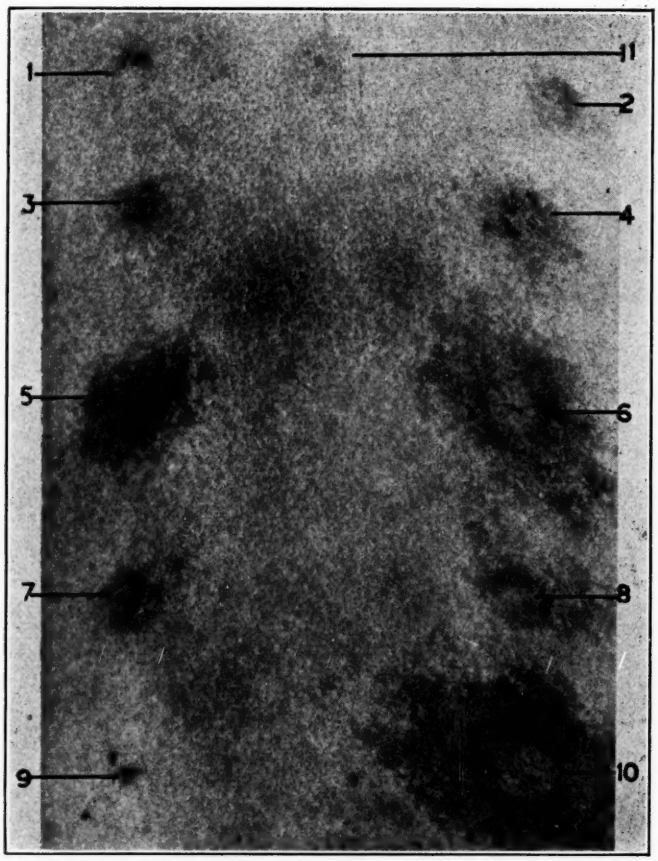

Fig. 4.

TABLE V

(Ba.-Cr. Series: 2)

March 18, 1935. All sites received 0.05 c.c. pollen fractions and saline as noted, with results as recorded.

LEFT				RIGHT			
SITE	PREVIOUS FRACTION	TODAY'S FRACTION	RESULT	SITE	PREVIOUS FRACTION	TODAY'S FRACTION	RESULT
1	D.	D.	Negative	2	D.	Whole extract	½ plus
3	S.D.	S.D.	? plus	4	S.D.	Whole extract	1 plus
5	P.D.	P.D.	1½ plus	6	P.D.	Whole extract	3 plus
7	C.A.	C.A.	½ plus	8	C.A.	Whole extract	1 plus
9	Whole extract	Whole extract	Negative	10	Saline	Whole extract	4 plus
11	D.	Whole extract	? (Normal serum control)				

All fractions were used in a dilution of 1:100

In Table V the resulting degrees of reaction are shown which followed the reinjection of one of each pair (the odd numbers on the left side) with the same fraction, and that which followed the injection of the even numbers of each pair (on the right side) with the whole extract. The results in sites 3, 5, and 7 show that all the reagin had not been utilized or exhausted, but at the same time greater reactions were obtained in the even numbers of each pair by the injection of the whole extract. The result in site 9 shows that the first injection of whole extract had exhausted all the reagins present. The reaction in site 10 shows the extent of reaction obtainable on this date with whole extract. Fig. 4 corroborates the reactions recorded in Table V.

TABLE VI

(Ba.-Cr. Series.) These results were not photographed.

March 20, 1935. Sites 1 to 8 and 10 received 0.05 c.c. fraction whole extract with results as recorded.

LEFT		RIGHT	
SITE	RESULT	SITE	RESULT
1	2 plus	2	Negative
3	1 plus	4	Negative
5	2 plus	6	Negative
7	½ plus	8	Negative
		10	Negative

The whole extract was diluted 1:100.

In Table VI the results are shown which followed the reinjection of all sites with whole extract. All the odd numbers (sites 1, 3, 5, and 7) show varying degrees of reaction; whereas their corresponding even numbers are negative. At this date it was not possible to take photographs of the results.

These results demonstrate the fact that, after each fraction or extract has united with or exhausted its respective and specific reagin present in the sensitized sites, the presence of other nonrelated antibodies can be demonstrated by the reinjection of the whole extract. *In other words, the antigenic constituents of a ragweed pollen solution are multiple as judged by the character of the antibodies present in the serum of a patient sensitive to ragweed.* The supposition of a single atopen in the pollen solution and a single reagin in the serum will not explain the results obtained.

Figs. 5, 6, and 7 and Tables VII, VIII, and IX (Nu.-Su. series) show the results obtained after each of the three reinjections made in the manner already outlined for this type of experiment.

In Table VII the primary preparation of sites 1 to 11 with sensitizing serum (Nu.) and site 12 with normal serum are recorded. The reactions resulting from injecting 4 fractions in pairs (1 and 2, 3 and 4, etc.) as well as whole extract, ragweed carbohydrate fraction (CHO) and

saline solution into sensitized areas are recorded in plus degrees of reaction. The fraction (whole extract) was injected into the normal serum control site. Fig. 5 shows the results. In site 9 the maximum

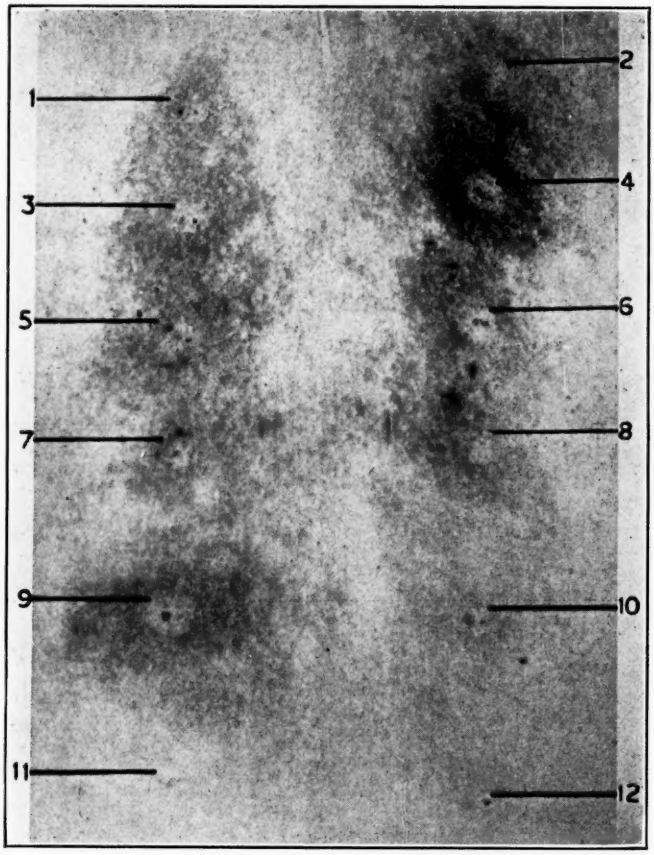

Fig. 5.

TABLE VII

(Nu.-Su. Series: 1)

March 25, 1935. Prepared sites 1 to 11 received 0.05 c.c. sensitizing serum. Prepared site 12 received 0.05 c.c. normal serum.

March 27, 1935. Prepared sites 1 to 12 received 0.05 c.c. pollen fractions and normal saline as noted, with results as recorded.

LEFT			RIGHT		
SITE	FRACTION	RESULT	SITE	FRACTION	RESULT
1	S.D.	3 plus	2	S.D.	3 plus
3	C.A.	4 plus	4	C.A.	4 plus
5	P.D.	3 plus	6	P.D.	3 plus
7	C.B.	2 plus	8	C.B.	2 plus
9	Whole extract	4 plus	10	Saline	Negative
11	CHO	Negative	12	Whole extract	Negative

The fractions used were diluted as follows:

C.B. and CHO	1:10
All others	1:100

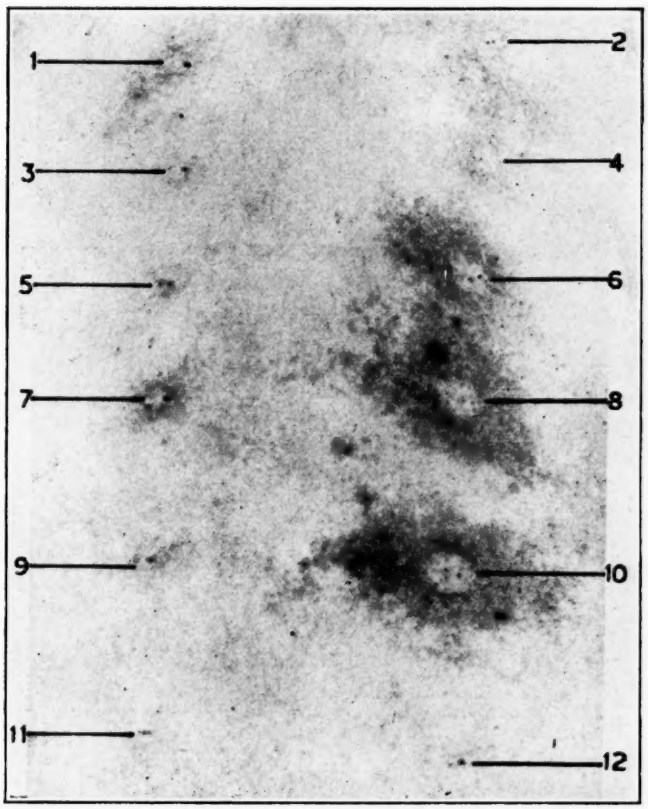

Fig. 6.

TABLE VIII

(Nu.-Su. Series: 2)

March 29, 1935. All sites received 0.05 c.c. pollen fractions and saline as noted, with results as recorded.

LEFT				RIGHT			
SITE	PREVIOUS FRACTION	TODAY'S FRACTION	RESULT	SITE	PREVIOUS FRACTION	TODAY'S FRACTION	RESULT
1	S.D.	S.D.	1 plus	2	S.D.	Whole extract	2 plus
3	C.A.	C.A.	Negative	4	C.A.	Whole extract	1 plus
5	P.D.	P.D.	Negative	6	P.D.	Whole extract	2½ plus
7	C.B.	C.B.	Negative(?)	8	C.B.	Whole extract	3 plus
9	Whole extract	Whole extract	Negative	10	Saline	Whole extract	4 plus
11	CHO	Saline	Negative	12	Whole extract	S.D. (normal saline control)	Negative

All fractions used were diluted as follows:

C.B. and CHO 1:10
All others 1:100

reaction induced by whole extract is shown. In site 10 the saline control is negative; site 11 injected with CHO is negative (for this case), and site 12 with whole extract in the normal serum control is also negative.

In Table VIII the reactions are shown which resulted from reinjecting the odd numbers (on the left side) 1 to 7 with the same amount of the fractions originally used, and the degree of reaction induced by

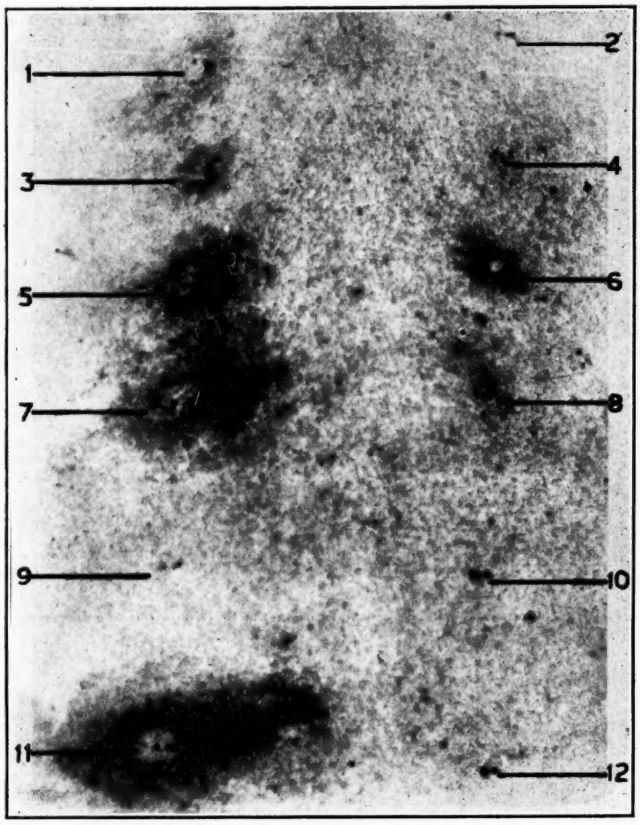

Fig. 7.

TABLE IX

(Nu.-Su. Series: 3)

March 30, 1935. Sites 1 to 8 and 11 received 0.05 c.c. fraction whole extract with results as recorded.

LEFT		RIGHT	
SITE	RESULT	SITE	RESULT
1	2 plus	2	Negative
3	½ plus	4	Negative
5	1½ plus	6	Negative
7	3 plus	8	Negative
9	------	10	------
11	4 plus	12	------

The fraction whole extract was diluted 1:100

injecting the even numbers (on the right side) with the whole extract. In only one odd numbered site (No. 1) was an appreciable reaction produced, but the corresponding site on the right side gave a greater reaction to the whole extract. Marked differences are shown between the reactions in sites 5 and 6 and between those in sites 7 and 8, the interpretation being that after these fractions had exhausted all their specific antibodies there still remained sufficient antibody to respond with a 2½ and a 3 plus reaction. Site 10 shows the extent of reaction obtainable on this date with whole extract. Sites 11 and 12 form two negative controls. Fig. 6 again closely corroborates the plus values given in the recorded results of Table VIII.

In Table IX the results are recorded following the injection of whole extract into sites 1 to 8 and site 11. As can be seen, all the even numbers on the right side are negative; whereas the odd numbers, 1, 3, 5, and 7 on the left side show varying degrees of reaction as recorded. The extent of reaction thus depicted in each odd number might be regarded as predictable from the extent of reaction obtained on the previous injections shown in Tables VIII and VII. Site 11 shows the available reagin still present at this date, six days after its introduction.

It should be mentioned that all photographs have been taken to scale and each reproduction therefore is strictly comparable with regard to the size of reactions in the same area on the back which has been used for all experiments of this type.

The third set (in this type of experiment) (Figs. 8, 9, and 10, and Tables X, XI, and XII, McL.-Cr. series) shows the results obtained after each of the three reinjections of antigenic fractions already outlined for this type of experiment.

In Table X the primary preparation of sites 1 to 11 with sensitizing serum (McL.) and site 12 with normal serum are recorded. It will be seen if Table VII is compared with Table X that the identical fractions in the same dilutions have been used in the same order to facilitate comparison of these two series. The reactions resulting from injecting 4 fractions in pairs (1 and 2, 3 and 4, etc.) as well as whole extract, ragweed carbohydrate CHO and saline solution into sensitized areas are recorded in plus degrees of reaction. The outstanding difference is that with this serum the fraction CHO gives a 4 plus reaction, whereas in the Nu.-Su. series it was negative as is well illustrated by Figs. 5 and 8 and Tables VII and X. It is also to be noted that the results with this fraction CHO in the original scratch skin tests Table I corresponds to the results with the Prausnitz-Küstner tests.

In Table XI the reactions are shown which resulted from injecting the odd numbers (on the left side) with the same amount of the fraction originally used, and the degree of reaction induced by injecting the even numbers (on the right side) with whole extract. With this serum all odd numbered sites were negative whereas all even numbered sites

showed varying degrees of reaction. This indicates as with the two other serums in this type of experiment that varying amounts of reagin still remained in the sensitized sites after each fraction had united with and exhausted its specific antibody.

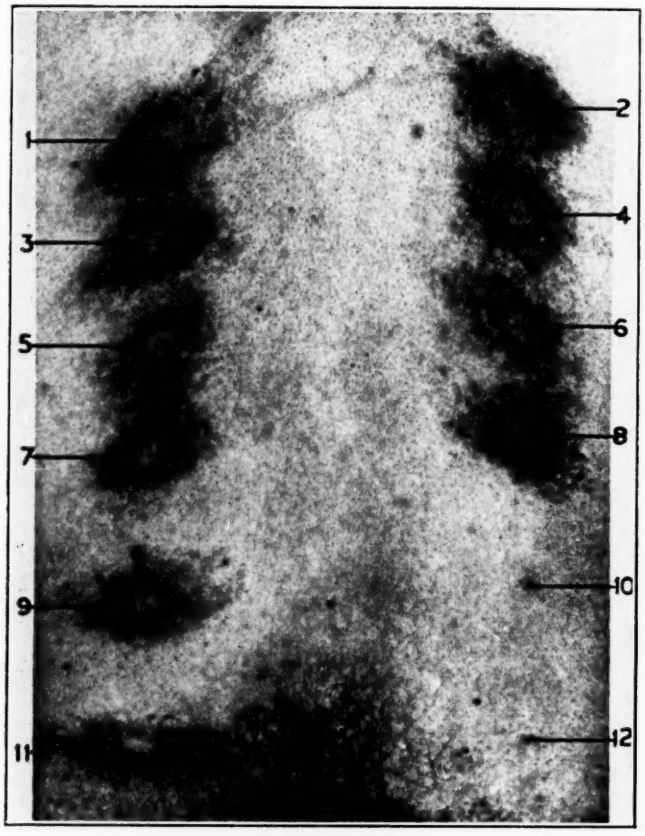

Fig. 8.

TABLE X

(McL.-Cr. Series: 1)

April 1, 1935. Prepared sites 1 to 11 received 0.05 c.c. sensitizing serum (McL.). Prepared site 12 received 0.05 c.c. normal serum.

April 3, 1935. Prepared sites 1 to 12 received 0.05 c.c. pollen fractions and normal saline as noted, with results as recorded.

LEFT			RIGHT		
SITE	FRACTION	RESULT	SITE	FRACTION	RESULT
1	S.D.	4 plus	2	S.D.	4 plus
3	C.A.	4 plus	4	C.A.	4 plus
5	P.D.	3½ plus	6	P.D.	3½ plus
7	C.B.	3½ plus	8	C.B.	3½ plus
9	Whole extract	4 plus	10	Saline	Negative
11	CHO	4 plus	12	Whole extract	Negative

All fractions used were diluted as follows:

C.B. and CHO	1:10
All others	1:100

Comparison of Tables VIII and XI shows the identical order has been adhered to except that in the McL.-Cr. series the fraction CHO was

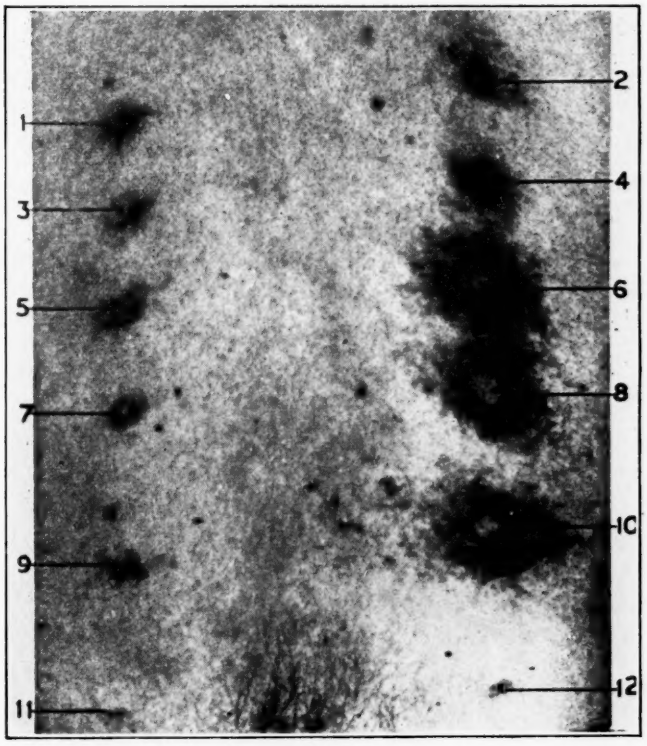

Fig. 9.

TABLE XI

(McL.-Cr. Series: 2)

April 5, 1935. All sites received 0.05 c.c. pollen fractions and saline as noted, with results as recorded.

LEFT				RIGHT			
SITE	PREVIOUS FRACTION	TODAY'S FRACTION	RESULT	SITE	PREVIOUS FRACTION	TODAY'S FRACTION	RESULT
1	S.D.	S.D.	Negative	2	S.D.	Whole extract	½ plus
3	C.A.	C.A.	Negative	4	C.A.	Whole extract	1 plus
5	P.D.	P.D.	Negative	6	P.D.	Whole extract	3-3½ plus
7	C.B.	C.B.	Negative	8	C.B.	Whole extract	3-3½ plus
9	Whole extract	Whole extract	Negative	10	Saline	Whole extract	4 plus
11	CHO	Saline	Negative	12	Whole extract (normal saline control)	CHO	Negative

All fractions used were diluted as follows:

C.B. and CHO	1:10
All others	1:100

injected into the normal serum control site 12. This was done to show a contrast to the positive result obtained in the sensitized site 11.

In Table XII the results are recorded following the injection of whole extract into sites 1 to 8 and 11. As can be seen, all the even numbers

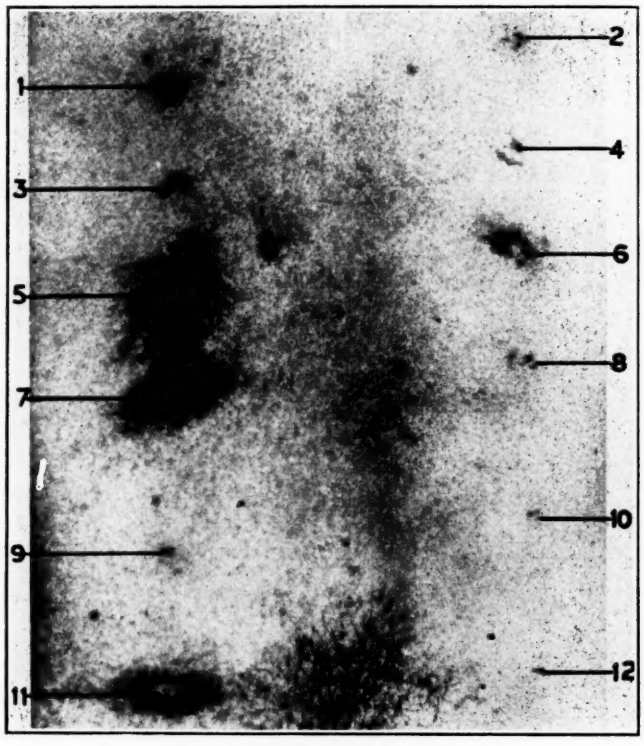

Fig. 10.

TABLE XII

(McL.-Cr. Series: 3)

April 6, 1935. Sites as noted received 0.05 c.c. fraction whole extract, with results as recorded.

LEFT		RIGHT	
SITE	RESULT	SITE	RESULT
1	½ to negative	2	Negative
3	Negative	4	Negative
5	2½ plus	6	Negative to ½ plus
7	2 plus	8	Negative
11	2½ plus		

The fraction whole extract was diluted 1:100

(on the right side) are negative with one doubtful exception, whereas all the odd numbers (on the left side) show varying degrees of reaction. Site 11 shows the available reagin still present after certain antibodies had been exhausted three days previously by the carbohydrate fraction.

Fig. 10 illustrates the plus values given in Table XII. These results corroborate the findings obtained in the two previous sets of experiments of this type, that there is more than one atopen in a pollen solution and more than one reagin in the serum of ragweed sensitive individuals.

In the original scratch tests with these fractions, Table I shows that the relative intensity of reaction induced by most of the fractions varied for each individual. Comparison of the results of the Prausnitz-Küstner tests in the Ba., Nu., and the McL. series shows there is also a considerable degree of relative variation for most of the fractions—that is greater than can be explained on the basis of experimental error. In the Nu. and McL. series the order and the dilution of the fractions have been made identical and thus are easily compared. The relative intensity of the resulting reactions is definitely different for the fractions P.D., C.B., and CHO. It is realized, however, that these experiments have not been designed to show this in an exact and quantitative fashion.

As a carbohydrate fraction labeled CHO has been used with the last two serums, some reference should be made to the rather unsettled position it occupies in the whole question of anaphylaxis or allergy. To show this it is frequently called a haptene. One of us (A. H. W. C.[1]) has previously reported that when a positive scratch test is obtained on ragweed hay fever cases with a carbohydrate fraction, a positive Prausnitz-Küstner reaction will also be obtained to the carbohydrate fraction; but that when the scratch test was negative the Prausnitz-Küstner reaction was also negative. This again is illustrated with the Nu. and McL. serums in this paper. Before more definite assertions can be made regarding its true rôle, the carbohydrate fraction must be nitrogen free; or it must be shown that the nitrogen is part of the carbohydrate molecule. Further work on this problem is in progress, and it is not the intention to make this subject an issue in this paper.

Results Obtained in the Third Type of Experiment by a Modification of the Prausnitz-Küstner Test.—Table II site 7 of the Nu.-Su. series shows that a 2 plus reaction was obtained with the fraction C.B. and the Nu. sensitizing serum which entirely or almost entirely exhausted its specific antibody as shown in Table VIII site 7. Table IX shows that there remained in this site 7 sufficient antibody to give a 3 plus reaction on the injection of whole extract.

TABLE XIII

ABSORPTION OF ANTIBODY BY C.B. ANTIGENIC FRACTION

April 3, 1935		
Mixture A	Nu. serum – 4 parts Plus C.B. (undiluted) – 1 part	Left at room temperature two and one-half hours
Mixture B	Nu. serum – 4 parts Plus NaCl solution 5% – 1 part	
Injection sites 1 and 2 (left side) received 0.05 c.c. Mixture A Injection site 3 (right side) received 0.05 c.c. Mixture B		

Table XIII (Nu.-Cr. series) shows the respective amounts of Nu. serum and the fraction C.B. that were used in the mixture labeled Mixture A. As undiluted C.B. fraction was made up in a 5 per cent NaCl solution, this strength of NaCl was used as a control and labeled Mixture B. These two mixtures were used as sensitizing mixtures. Mixture A was injected into sites 1 and 2 on the left side and Mixture B (control) into site 3 on the right side.

Table XIV shows that site 1 (sensitized by Mixture A) gave a 3 plus reaction to whole extract, whereas site 3 (also sensitized by Mixture A) failed to react to the fraction C.B. The control site 3 (sensitized by

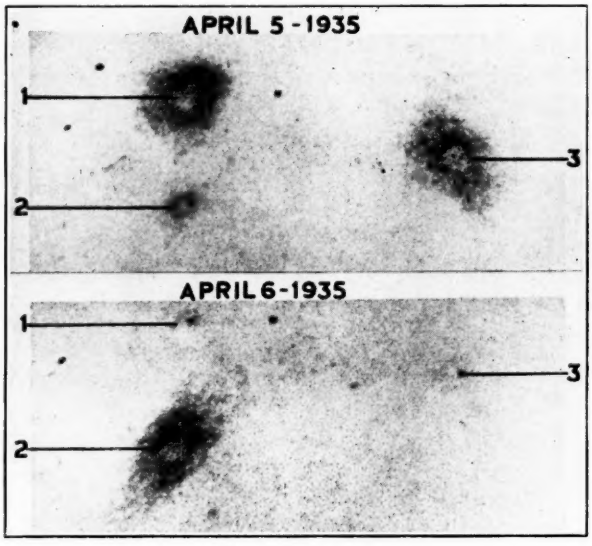

Fig. 11.

TABLE XIV

ABSORPTION OF ANTIBODY BY C.B. ANTIGENIC FRACTION

April 5, 1935	
Sites 1 and 3 received 0.05 c.c. whole extract 1:100	
Site 2 received 0.05 c.c. C.B. 1:100	
RESULTS	
LEFT	RIGHT
Site 1—3 plus	Site 3—3½ plus
Site 2—negative	Control site—7 (Table VII)

April 6, 1935	
Sites 1, 2, and 3 received 0.05 c.c. whole extract 1:100	
RESULTS	
LEFT	RIGHT
Site 1—negative	Site 3—negative
Site 2—2½ plus	

Mixture B) gave a 3½ plus reaction to whole extract. Twenty-four hours later, April 6, the reinjection of whole extract in all three sites showed that there remained in site 2 sufficient antibody to give a 2½ plus reaction, whereas no result was obtained in sites 1 and 3. Fig. 11 illustrates graphically what Table XIV records in plus values.

This modification of the Prausnitz-Küstner reaction supports our interpretation that after one allergically active fraction has united with all its available antibody there remain other antibodies whose presence can be demonstrated by injecting the whole extract. *In other words, the atopens are multiple as are also the reagins in an individual serum.*

THE METHODS OF PREPARATION AND FRACTIONATION OF RAGWEED EXTRACT

The air-dried pollen was roughly defatted with anhydrous ether at room temperature for a few hours, and the ethereal extract was removed by centrifugation. The pollen residues freed from ether by a current of air were then ground in a ball mill for eight hours. This treatment ensures disruption of the individual pollen grains, thereby aiding subsequent extraction. In this particular experiment the extraction was carried out in the ball mill, which was housed in a refrigerator operating at 40° F. For every 5 grams of original ragweed pollen 100 c.c. of a 5 per cent solution of sodium chloride were added, together with a small amount of chloroform to act as preservative and help to complete the removal of any remaining fatlike substances. The extraction period was about fourteen hours. The extract was centrifuged at fair speed for half an hour, the resulting supernatant fluid being cloudy opalescent in appearance. This solution we designate a 5 per cent extract of ragweed pollen in 5 per cent sodium chloride solution, referred to briefly as the original solution (or O).

One hundred c.c. of this original solution were set to dialyze through cellophane tubes into several changes of distilled water over a period of twenty-four hours. It may be mentioned here that throughout these fractionation experiments all solutions were preserved with chloroform, until finally sterilized by Seitz filtration. A precipitate of proteinlike material settled out during the dialysis. This precipitate was removed at the end of the dialysis by filtration and the filtrate (125 c.c.) containing the nondialyzable constituents of the extract treated with sufficient sodium chloride to give a 5 per cent solution. This solution is referred to as the nondialyzable fraction, or D, of the ragweed extract. It should be noted that this solution also lacks those substances (the precipitate mentioned above) soluble in 5 per cent sodium chloride solution, but insoluble in water.

Another 100 c.c. portion of the original solution was treated with glacial acetic acid to give a reaction of about pH 4.0. An equal volume (100 c.c.) of saturated ammonium sulphate solution was added and the

mixture allowed to stand in the refrigerator for half an hour. It was then centrifuged; and the opalescent supernatant fluid so obtained was filtered and set to dialyze immediately in cellophane tubes, against running tap water. As soon as possible the precipitate in the centrifuge cups was ground up with 5 per cent sodium chloride solution together with a small amount of a very dilute solution of sodium hydroxide added in just sufficient amount to give a mildly alkaline reaction. This solution was set to dialyze immediately. Both solutions were dialyzed into running tap water for forty hours, then into several changes of distilled water for twelve hours. Small amounts of deposits were removed by filtration. Sodium chloride was added to each to give a 5 per cent solution. The solution called the ammonium sulphate supernatant, or more briefly S.D., occupied a volume of 420 c.c., while the ammonium sulphate precipitate, or P.D., solution measured 35 c.c.

The charcoal used was Norite A. Immediately before use, a sample was well washed with water, filtered off and dried in an oven and finally heated in an evaporating dish over a Bunsen burner. Two gm. of this material were added directly to 85 c.c. of the cold original extract. After shaking, the solution was returned to the refrigerator for four hours; the flask being shaken from time to time. It was then filtered. The filtrate was perfectly clear and colorless. This solution was labeled Charcoal A, or C.A. The charcoal residues were not washed, but were shaken immediately with 90 c.c. of a 2 per cent solution of disodium hydrogen phosphate crystals ($Na_2HPO_4 12\ H_2O$). The mixture stood at air temperature for three hours with occasional shaking, then in the refrigerator overnight. The following morning the insoluble material was removed by filtration, a perfectly clear filtrate resulting. This solution was neutralized by the addition of a little hydrochloric acid and about 2.5 gm. of NaCl were then added. This eluted solution is referred to as Charcoal B, or C.B.

The procedure adopted in the preparation of the different batches of whole extract was almost identical with that described above for the 5 per cent saline extract; except that distilled water was used instead of the saline solution.

ISOLATION OF CARBOHYDRATE FRACTION

Seventy grams dried pollen were roughly defatted with anhydrous ether, as above. The material was then extracted with 2 liters distilled water at 37° C. for ten days, chloroform being used as preservative. The chloroform also helped by further defatting the pollen. The material was centrifuged and filtered. Acetic acid was added to give a pH of about 4.2; this resulted in a precipitation of protein which readily flocculated. The precipitate was later separated on the centrifuge and was worked up separately. After separation of the protein precipitate

(pH 4.2) the extract was treated with basic lead acetate. The resulting precipitate was removed, more lead acetate added, and the solution made definitely alkaline to thymol blue by addition of potassium hydroxide. The lead hydroxide precipitate so produced carried down with it the bulk of the sugar material. The precipitate was collected on a large Buchner funnel, washed with a little very dilute potassium hydroxide solution, transferred to an ice-cold mortar, and dissolved by grinding in ice-cold dilute sulphuric acid. The lead sulphate was removed on the centrifuge. The clear supernatant fluid was treated with the exact amount of barium hydroxide to remove excess sulphuric acid. After removal of barium sulphate the solution was neutralized to pH 7.0 with potassium hydroxide and concentrated to low bulk under reduced pressure at 40° C. The concentrate was set to dialyze, using cellophane tubes. The contents of the dialyzing sac were reconcentrated and then precipitated by addition of several volumes of alcohol. After standing in the cold overnight the precipitate was collected, redissolved in distilled water, freed from insoluble material, and reprecipitated by addition of absolute alcohol. All these operations were conducted in the centrifuge tubes, thus conserving the amount of material. The precipitate was redissolved in a small amount of ice-cold 5 per cent trichloracetic acid; insoluble material was removed, and the solution again treated with absolute alcohol. Again the precipitate was dissolved in water and reprecipitated by addition of absolute alcohol. The precipitate was then washed with alcohol and ether, and dried.

The final product, so obtained, was a slightly colored powder (yield approximately 2 per cent original pollen material), slightly hydroscopic, readily soluble in water to give a clear solution. It gave a strongly positive Bial reaction. On acid hydrolysis it yielded 58 per cent reducing sugar (Shaffer-Hartman) calculated as glucose. The products of hydrolysis are not fermented by yeast, showing absence of glucose, fructose, and mannose, but the positive Bial test indicates the presence of pentose sugar or uronic acids. An osazone was prepared from the hydrolyzed products, but it could not be identified. The dry material contained 1.5 per cent total nitrogen. Further purification by repeated precipitation of some of the material from aqueous solution by addition of absolute alcohol indicated that the nitrogen content of the material could be further reduced. Work on this problem is still in progress.

NITROGEN DETERMINATIONS

All the nitrogen estimations have been made by the micro-Kjeldahl method. The protein nitrogen values were obtained by difference, that is to say, the total nitrogen contents of the solutions were determined before and after precipitation with trichloracetic acid. Sufficient trichloracetic acid was added to give a 5 per cent solution, and the mixtures

were allowed to stand at room temperature overnight. It is assumed that under these conditions the protein is completely precipitated unaccompanied by nitrogenous substances of smaller molecular weight.

TABLE XV

SOLUTION	TOTAL N	PROTEIN N
	MG. PER C.C.	MG. PER C.C.
Fraction O	0.630	0.080
Fraction D	0.169	0.025
Fraction P.D.	0.063	Very slight precipitate. Too small to obtain an accurate figure
Fraction S.D.	0.078	No precipitate. Not even an opalescence
Fraction C.A.	0.382	Too small to determine
Fraction C.B.	0.023	Too small to determine
Whole extract 334: 5% (used for scratch tests)	0.57	0.08
Whole extract 1134: 2.5%	0.297	Too small to determine
CHO (solid) 1.5% total nitrogen		

Dilutions of all solutions were based on the amount of original extract used in their preparation. Thus 10 c.c. of a 1:10 dilution of the different fractions were made in the following manner:

O	1.0 c.c. O + 9.0 c.c. water
D	1.3 c.c. D + 8.7 c.c. water
S.D.	4.3 c.c. S.D. + 5.5 c.c. water
P.D.	0.3 c.c. P.D. + 9.7 c.c. water
C.A.	1.0 c.c. C.A. + 9.0 c.c. water
C.B.	1.0 c.c. C.B. + 9.0 c.c. water
Whole extract 5%	1.0 c.c. + 9.0 c.c. saline
Whole extract 2.5%	2.0 c.c. + 8.0 c.c. saline
Carbohydrate fraction:	125 mg. of material were dissolved in 50 c.c. normal saline.

DISCUSSION

We feel that the experimental results presented in this paper prove conclusively that there is a multiplicity of allergically active substances in ragweed pollen solution, and further that there is more than one specific reagin in the serum of the individual ragweed hay fever subject. We suggest that these facts have an important bearing among others on the three following aspects of pollen therapy:

A. No wholly acceptable method of standardization of pollen solutions has yet been proposed. The three most widely known and adopted methods are those based on the weight of original pollen per volume of extracting fluid (which is the basis adopted for dilution of the six fractions and other solutions except the carbohydrate fraction used in these experiments), the total nitrogen content of the extract, and the protein nitrogen content of Cooke and Stull. We have no intention of suggesting yet an-

other method, but we do suggest that the data accumulated in our studies indicate that both types of nitrogen content methods may be misleading.

In particular we wish to draw attention to the considerable differences in nitrogen contents, total and protein, of the two fractions O and D. Fraction O contained 0.65 mg. and 0.08 mg. per c.c. of total and protein nitrogen respectively. Fraction D contained 0.169 mg. and 0.025 mg. per c.c. of total and protein nitrogen respectively. Despite this difference in the nitrogen contents, the fraction D gave greater reactions in the Prausnitz-Küstner tests of the first type of experiments in three of the five cases: in two it was approximately equal though with one of them reinjection of whole extract favored the greater value of the fraction D. One of these serums was used in repeat experiments with identical results. Table I, which gives the results of original scratch tests, corroborates these results obtained by Prausnitz-Küstner tests.

B. Again, considerable attention has been focused on the identity or otherwise of the allergically active substances in giant and low ragweed. Quite frequently those engaged in attempts to solve this problem tacitly assume singleness of atopic content of the two types of pollens. It seems to us that proof of the theory, that in pollen solutions we have to deal with a multiplicity of allergically active constituents, must be taken into consideration before convincing conclusions can be drawn.

C. In 1922 one of us[2] reported that about 90 per cent of a relatively small number of patients received effective protection treated with what amounted to three types of solution of ragweed pollen. Belief on empirical grounds in the therapeutic value of multiple solutions of a single pollen led to the production of several fractions of more or less definite chemical characteristics. These results were reported upon in 1926,[3] and since then at least four fraction solutions have been used for ragweed pollen in the desensitization of patients. Some time after the therapeutic use of these various fractions (based upon the differential results of the skin tests), an approximate survey of the results on a considerable number of patients showed that over 95 per cent received a favorable and satisfactory-to-the-patient degree of protection. This is a very high percentage of protection as judged by reports in the literature and is seemingly to be attributed to the use of selected multiple solutions.

CONCLUSION

By means of the Prausnitz-Küstner reaction and an in vitro absorption modification of this reaction, we have shown that there is more than one allergically active substance in ragweed pollen solution and further that in the serum of the ragweed hay fever individual there is more than one specific reagin. By the same tests we find that the part played by one allergically active substance varies considerably in different patients,

as judged by the quantitative amount of the different specifically active reagins present in the serum of each patient.

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DISCUSSION

DR. MATTHEW WALZER, BROOKLYN.—I do not wish to enter into a discussion on the question of pollen fractions, but I should like to issue a note of warning on the technic used for experiments in passive transfer. It is a rather dangerous procedure (dangerous as far as results to be obtained or conclusions to be drawn are concerned) to use one recipient for simultaneous testing with many fractions and many antigens. You must consider the fact not only that there is neutralization at the point of testing, but also that there is considerable absorption and that there is neutralization at distant sites. Therefore, when you inject a test into a sensitized site, and a control, you are injecting into the skin substances which neutralize not only that site but, to some extent, distant sites as well. That is the first source of error and something that must be taken into consideration when you are doing more than one test on a passively sensitized subject. Another point which is even more important is that a passively sensitized site which has been tested is a very poor site for further testing. That was the fact that prompted Miss Bowman and myself to develop the technic of neutralization in vitro in preference to neutralization in the skin. When there is a reaction on a passively sensitized site (and in this case, very marked reactions were obtained), that site is almost worthless for retesting. I would warn those who are using this technic for this type of study to be careful to take into consideration damage to remaining reagins as well as refractoriness of tissues at the seat of a positive reaction on a passively sensitized site. I really believe the only way this problem can be studied is by the technic of neutralization in vitro.

DR. BEN Z. RAPPAPORT, CHICAGO.—The results of Dr. Caulfeild's report surprise me because of their specificity. In animal experimentation, using the precipitin reaction as a means of judging the specificity of albumin and globulin fractions of the giant and short ragweed pollens, we found no such similarity of specificity.

In 1930, we worked with the short ragweed pollen, fractionating it by various concentrations of ammonium sulphate. We obtained a globulin, an albumin, and a pseudoglobulin, the latter mainly a mixture of albumin and globulin. Using immune precipitin serum prepared in rabbits against short ragweed albumin, we failed to find any specificity of the fractions of this same pollen. In addition, using various other related pollens, as burweed, slender ragweed, cocklebur, southern ragweed, and giant ragweed, we found that the precipitin reaction with the serum prepared against short ragweed albumin was positive in approximately the same titer for all. Not only that, but absorption experiments, using

albumin and globulin, failed to show any specificity of the fractions prepared from short ragweed.

This is quite in line with the experience of protein chemists with vegetable proteins. They are not so specific as the animal proteins are, at least in animal experimentation. This, of course, may not apply to the Prausnitz-Küstner method of antigen differentiation.

DR. JOSEPH HARKAVY, NEW YORK.—How sure is Dr. Caulfeild that he is dealing with pure fractions? How sure is he that some of his fractions have not carried over to the globulins or albumins of another fraction?

DR. F. M. RACKEMANN, BOSTON.—Dr. Caulfeild does not need any defense, I am sure of that, but I should like to say that during the past two years, first Dr. Simon, and more recently, Dr. Wagner have been working extensively with this Prausnitz-Küstner general method of active desensitization and find it works well on studies of specificity of preparations. It seems to me that Dr. Caulfeild's paper is the sort of work that gets us ahead, and I congratulate him.

DR. GEORGE PINESS, LOS ANGELES.—Apparently, Dr. Rackemann has forgotten that Dr. Cohen and Dr. Bernton as well as Dr. Caulfeild in earlier experiments have done this same type of work in separating pollen fractions and they got nowhere with it. Then again, Dr. Caulfeild, in discussing whole pollen, said he used distilled water which we all know is a very good extractive for obtaining protein from pollen in solutions. The best extractive of which we know is a buffer salt, and I doubt very much whether the reaction that Dr. Caulfeild showed as negative indicated an absence of protein; at least, he did not bring this out in his talk about the protein nitrogen. The most satisfactory preparation for treatment is, after all, the whole pollen grain properly extracted.

DR. CAULIFEILD.—Dr. Walzer pointed out that a site once used for passive transfer was damaged. That is perfectly true. I mentioned that we did nine sets of tests with the five serums, and we discovered how extensively damaged a site could become in the early tests. In the pictures I showed, which are very clear, all sites were new with the exception of those on the arm. Another point as regards the damaged site is this: If you use all the fractions, or something that has all the fractions in it, you do less damage. In those sites in which reagins have not been taken out or exhausted, the damage is more marked.

Dr. Rappaport spoke about precipitins. We did not consider this type of experimentation applicable to the problems we wished to solve. The Prausnitz-Küstner test seemed the most suitable—or the only one perhaps for the problems in view.

I shall attempt to answer Dr. Harkavy's questions in the following manner: Our conception has been that it is impossible, at least at the present time, to obtain active fractions of chemical purity. The fractional solutions, however, were prepared in such a manner that the proportional distribution of the different allergically active substances varied considerably in the different solutions. This proportional distribution varied sufficiently in some of the solutions that in a sense they could be regarded as etiologically different antigens. As a hypothesis let us grant that solution I contains atopens A and B, but A is present in close to 90 per cent, i.e., solution I has all of atopen A extractable, whereas atopen B is present in but a small or negligible percentage. In contrast to solution I,

solution II has the atopen B in close to 90 per cent and but a negligible quantity of atopen A. Such a contrasting proportionality of allergically active substances will, I hope, help one to visualize the conception which the results of the Prausnitz-Küstner tests have demonstrated.

I wish to thank Dr. Rackemann for his remarks of encouragement. The results which he mentions with regard to detectable differences between the solutions of different grasses by means of the Prausnitz-Küstner tests interest me extremely. I hope he interprets his results, from a therapeutic point of view, as indicating the value of using multiple grass solution rather than a single solution such as a solution of timothy pollen. I fear I digress and go beyond the scope of his remarks with regard to the identity or not of the different grass pollen. Our members can, I believe, be divided into two groups, those who thoroughly believe in the therapeutic value of using the different grass pollens in chronological order according to the results obtained by testing, and those who feel one grass pollen solution or perhaps two meet the requirements because any way there is no difference between any of them. I hope Dr. Rackemann's results will aid in settling this issue. My own position is, I hope, clear.

What Dr. Piness says about the value of buffered saline is true. He will see that we have used his own fluid for extracting pollen, and this formed one of the solutions used as an additional control. The results are recorded in Table I, which it was impossible to present today because of lack of time. We would like to stress that we were not attempting to determine the best extract nor necessarily the most potent. The problem we had in view was different from that and is perhaps exemplified in the answer I have already given to Dr. Harkavy's remarks. The method of grinding which we use disrupts the pollen grains to an amorphous mass as demonstrable by microscopic examination. The extracts made in distilled water were found to be very potent but must be fresh and preserved in a refrigerator. Dr. Piness mentioned Dr. Cohen and Dr. Bernton's work. He must, I think, mean Dr. Cohen who was associated with both myself and Dr. Eadie in what was, I believe, the original work along these lines. We all felt we had got somewhere with that piece of investigation, and I know Dr. Cohen, now Dr. Markowitz, still thinks so. At any rate it was my belief in these results in 1926 that led to our taking up the problem again along present lines of investigation. Dr. Bernton a year or so later, using different methods, and as well working with timothy and not ragweed pollen, felt, I believe, that he "got nowhere."

LOCAL SERUM SICKNESS IN MAN FOLLOWING THE INTRACUTANEOUS INJECTION OF SMALL AMOUNTS OF ANTISERUMS*

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RECORDED observations of local serum sickness following a single small intracutaneous injection of a foreign serum are infrequent. Moss was apparently the first to observe such a reaction. Three of thirty individuals who had been inoculated intracutaneously on the volar aspect of the forearm with 0.01 c.c. of normal undiluted horse serum developed an urticarial wheal, erythema, and itching at the site of injection six to twenty-four hours later. The reaction lasted about one to three days. All three of these individuals had received an immunizing course of diphtheria toxin-antitoxin injections some years previously. Hooker reported a comparable local reaction in a woman who was similarly injected with 0.1 c.c. of normal horse serum diluted 1:10. Twenty minutes after the injection a wheal, 1.2 cm. in diameter, associated with erythema and itching, appeared at the site of injection. This reaction disappeared in an hour but was followed in five and twelve hours with similar reactions of greater intensity. Hooker, believing that these three separate reactions might be due to multiple sensitization to the protein fractions of horse serum, fractionated the serum into pseudoglobulin, euglobulin, and albumin. These antigens tested on the same patient each produced but one reaction; the pseudoglobulin reaction appeared early corresponding to the first wheal and erythema reaction in response to the whole serum, while the time interval for the appearance of the euglobulin and albumin fraction reactions corresponded to the second and third reactions following the injection of horse serum. Rabbit serum similarly injected produced no reaction. Tuft has also observed a reaction "resembling a local serum sickness" in six of eighteen subjects who were inoculated with even smaller amounts of horse serum. The one reaction described appeared three days after injection and consisted of swelling, erythema and itching.

We have been interested in the differences which serums from various species of animals possess in their ability to incite serum sickness. One of us (D. K.) has found that the injection of horse serum

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in rabbits, alone of all the serums tested, produced a reaction which Fleischer and Jones have characterized as serum sickness. Sheep serum, dog serum, guinea pig serum, and human serum failed to produce any symptoms when injected in rabbits. This fact suggested that horse serum might be almost unique in its capacity to incite the distressing symptoms of serum sickness. Considerable evidence exists in the literature that not all serums are alike in their ability to induce serum sickness in man. For example, Kraus and his coworkers found that only a small percentage of people developed serum sickness following treatment with immune serum derived from cattle. Kyes and Carey have failed to observe serum sickness following therapeutic treatment of man with fowl serum.

The production of local serum reaction suggested itself as a possible method for carrying out a comparative study in man, of the ability of a number of foreign serums to produce serum sickness. Serums from four species of animals which had received immunizing injections of a bacterial antigen were chosen. Purified antibody solutions (globulin fraction of serum) from two of the animal species were also used.

MATERIALS AND METHODS

The serums used for the tests were as follows: Horse antipneumococcus Type I serum obtained from the New York City Board of Health; sheep antihemolytic streptococcus serum obtained from Dr. Coburn; rabbit antipneumococcus Type I serum prepared in our laboratory; and bovine diphtheria antitoxin obtained through the courtesy of the Mulford Laboratories. The Mulford Laboratories also supplied equine and bovine tetanus antitoxin globulin.* The subjects of the tests were twelve volunteers, five men and seven women, of this department. All were in their twenties or thirties. Four had received horse serum either during immunization against diphtheria or in the treatment of this disease some years previously. Five were subject to allergic reactions from one or more antigens (see Table I). One-tenth of a cubic centimeter of undiluted serum was used for each test, except for one subject, M. J., who received 0.1 c.c. of a 1:10 dilution of the horse and rabbit serums. The horse serum was injected intracutaneously in the volar surface of the left forearm and the rabbit serum in the right forearm. Two months later the sheep serum was injected in the left forearm and the bovine globulin in the right forearm. The bovine serum and horse globulin were injected into the left and right forearms, respectively, six months later. Only ten of the twelve subjects were available for this last test.

*The globulin solutions had a protein content more than twice that of the serum.

RESULTS

The results are presented in Table I and Chart 1. The most extensive and frequent local serum sickness followed the injection of sheep serum, although horse serum proved to be a close second in its ability to incite a severe local reaction. The reactions obtained from bovine serum were as frequent in occurrence as those from sheep serum but were on the whole very much milder. Rabbit serum produced local serum sickness of mild degree in only one individual. The purified horse globulin proved very much less active in causing a local reaction than did the horse serum, while the purified bovine globulin was almost completely inactive in causing a local reaction. The details concerning the type and duration of the reaction to each foreign protein follow.

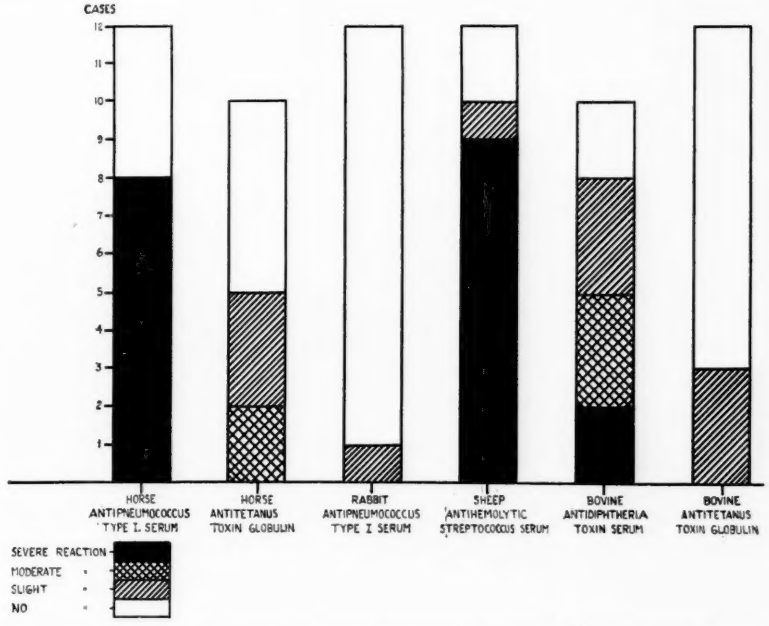

Chart 1.—Degree of local serum sickness produced by the intracutaneous injection of horse, rabbit, sheep, and bovine serum, and horse and bovine globulin.

Horse Antipneumococcus Type I Serum.—Except for a mild erythema no immediate reaction was observed following the intracutaneous injection of 0.1 c.c. of horse serum. Within twenty-four hours, however, six of the twelve subjects had developed multiple wheals which extended for an area of about 5 cm. around the site of injection and which varied from 2 mm. to 3 mm. to 2 cm. to 3 cm. in diameter. These were associated with marked erythema and itching. The wheals came and went, at times leaving the arm perfectly normal in appearance and then returning in added numbers. Two of the reactors developed the first wheals about two hours after injection, two others in about

TABLE I

INDIVIDUAL HISTORIES OF LOCAL SERUM SICKNESS FOLLOWING THE INTRACUTANEOUS INJECTION OF 0.1 C.C. OF FOUR FOREIGN ANTISERUMS AND TWO PURIFIED ANTIBODY SOLUTIONS IN TWELVE NORMAL ADULTS

SUBJECT	PREVIOUS HORSE SERUM INJECTIONS	ALLERGIC DISEASES	REACTION TO					
			HORSE ANTIPNEUMOCOCCUS TYPE I SERUM	HORSE TETANUS ANTITOXIN GLOBULIN	RABBIT ANTIPNEUMOCOCCUS TYPE I SERUM	SHEEP ANTISTREPTOCOCCUS SERUM	BOVINE DIPHTHERIA ANTITOXIN SERUM	BOVINE TETANUS ANTITOXIN GLOBULIN
G. H.	Diphtheria toxin-antitoxin	Constitutional reaction to salmon	Wh., eryth. and itch. 4th to 8th day	Eryth. and itch. 48th hour	Negative	Wh., eryth. and itch. 2nd to 3rd day	2 wh., eryth. and itch. 36 hours. Eryth. and itch. 2nd day	Wh., eryth. and itch. for 1 hour 5th day
A. O.	Antitoxic horse serum	None	Wh., eryth. and itch. 2nd to 16th hour and 4th to 6th day	Not tested	Negative	Itch. 6th, 7th, and 8th days	Not tested	Negative
J. M.	None	None	Negative	Negative	Negative	Wh., eryth. and itch. 3rd hour to 6th day	Wh., eryth. and itch. 10th, 18th, 26th, 40th, 70th hours	Negative
C. J.	None	Hay fever and dermatitis to shellfish	Wh., eryth. and itch. 16th hour to 5th day	Wh., generalized edema 6th hour. Eryth. and itch. 7th day 1 hour	Negative	Wh., eryth. and itch. 6th to 12th day	Wh., eryth. and itch. 5th, 24th and 29th hours	Negative
S. K.	Antitoxic horse serum	Hives in 1926	Negative	Negative	Negative	Wh., eryth. and itch. 4th to 6th day	1 wh. 100th hour	Negative

Wh. = wheal.
Eryth. = erythema.
Itch. = itching.

TABLE I—CONT'D

SUBJECT	PREVIOUS HORSE SERUM INJECTIONS	ALLERGIC DISEASES	REACTION TO					
			HORSE ANTI-PNEUMOCOCCUS TYPE I SERUM	HORSE TETANUS ANTITOXIN GLOBULIN	RABBIT ANTI-PNEUMOCOCCUS TYPE I SERUM	SHEEP ANTI-STREPTOCOCCUS SERUM	BOVINE DIPHTHERIA ANTITOXIN SERUM	BOVINE TETANUS ANTITOXIN GLOBULIN
J. C.	None	None	Wh., eryth. and itch. 2nd hour to 4th day	1 crop large wh., eryth. and itch. 2nd day	Wh., eryth. and itch. 2nd to 16th hour	Wh., eryth. and itch. 6th hour to 2nd day	Wh., eryth. and itch. 3rd and 20th hour	Negative
F. O.	None	None	Wh., eryth. and itch. 6th and 7th days	Not tested	Negative	Wh., eryth. and itch. 3rd hour to 4th day	Not tested	Eryth. and itch. 1 hour 6th day
G. F.	None	Dermatitis to mercury	Negative	Negative	Negative	Negative	Negative	Negative
L. B.	None	Hay fever to guinea pig and rat dander	Wh., eryth. and itch. 4th hour to 2nd day	Negative	Immediate wh., eryth. and itch. 1 hour	Wh., eryth. and itch. 16th hour to 7th day	1 small wh., 175th hour	Negative
M. J.	Antitoxic horse serum	None	Wh., eryth. and itch. 4th hour to 3rd day	Wh., eryth. and itch. 5th hour to 3rd day	Negative	Wh., eryth. and itch. 4th hour to 7th day	Wh., eryth. and itch. 4th hour through 6th day. 1 wh. 9th day	Negative
B. S.	None	None	Wh., eryth. and itch. 16th hour to 6th day	Wh., eryth. and itch. 18th, 24th, 48th hours and 6th, 8th day	Negative	Wh., eryth. and itch. 5th hour to 6th day	Wh., eryth. and itch. 2nd to 8th day	1 wh., eryth. and itch. 1 hour 7th day
D. K.	None	Hay fever and asthma to ragweed pollen	Negative	Negative	Negative	Negative	Negative	Negative

four hours, while the remaining two did not show a reaction until the following morning, about sixteen hours after injection. In one of the reactors, A. O., the reaction had disappeared on the following morning, and the arm remained normal in appearance until the fourth day. At this time small scattered wheals associated with erythema and itching made their appearance again and came and went during the next

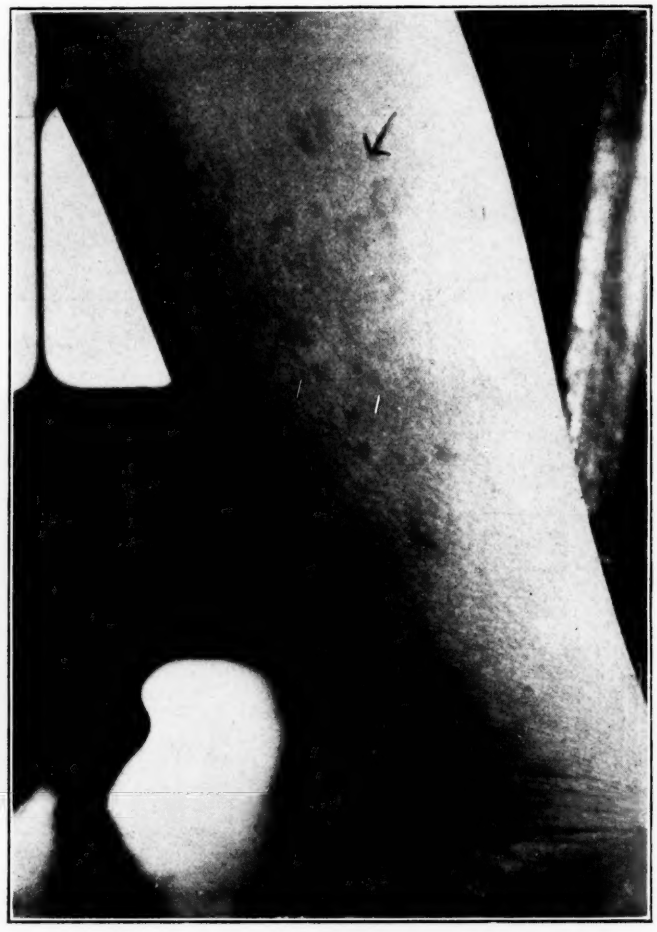

Fig. 1.—The forearm of M. J. on the sixth day after the intracutaneous injection of 0.1 c.c. of sheep antihemolytic streptococcus serum at the tip of the arrow. Note the wheals and erythema which were accompanied by severe itching.

four days. Succeeding crops of wheals tended to arise further from the site of injection, especially toward the dependent, distal portion of the forearm. Wheals, however, might recur in the same site. The remaining five subjects who showed this very accelerated type of local serum sickness reacted continuously for two to six days. The itching was sufficiently severe to interfere sometimes with sleep. The size of the wheals varied greatly within the same person both during

a single attack and in different attacks. No portion of the day was exempt from attacks, but the reactions seemed to be somewhat more severe in the morning and evening. Fig. 1 illustrates such a local serum sickness reaction. This reaction was elicited by sheep serum, but it is also typical of the reaction produced by horse serum.

Of the remaining six people inoculated with horse serum two showed no reaction for several days. On the fourth day after injection, as shown in Table I, G. H. developed wheals, erythema, and itching for the first time. This reaction was like those already described. The wheals were of variable size and were distributed at a distance of several centimeters from the site of injection. They came and went for a period of three days. The fourth day was characterized by erythema and itching without visible wheal formation. F. O. developed no reaction until the beginning of the sixth day. The wheals developed in the evening, and the associated itching was so severe as to interfere with sleep. The following day small wheals associated with large areas of erythema and itching still persisted. The remaining four subjects developed no true local serum sickness following the injection of horse serum. J. M., however, had slight erythema and itching at the site of the horse serum injection on the eighth day after injection.

In summary, the intracutaneous injection of 0.1 c.c. of undiluted horse antipneumococcus Type I serum resulted in severe local serum sickness in eight of twelve subjects tested. This serum sickness was characterized by recurrent wheals, erythema, and itching which tended to occur farther and farther from the site of injection; however, it might recur in the same area or directly in the site of injection. At one time almost the entire volar surface of the forearm might be involved, but the picture was a constantly changing one, and a reaction such as that shown in Fig. 1 might disappear in an hour and be followed shortly by a single wheal only 2 mm. in diameter surrounded by 2 cm. or 3 cm. of erythema and itching intensely. In six of the eight positive reactors the reaction appeared within twenty-four hours of the injection and lasted two to eight days. In the remaining two the reaction appeared on the fourth and sixth days after injection and lasted respectively four and one days.

Horse Tetanus Antitoxin Globulin.—In contrast to the horse serum the horse globulin produced a milder and briefer local serum sickness. Five of the ten subjects reacted, one, G. H., with only one bout of of erythema and itching, while two others, J. C. and C. J., had only one and two brief reactions respectively. M. J. and B. S. had more protracted and repeated attacks, though milder than those produced by the whole serum. The horse globulin was therefore on the whole much less provocative of local serum sickness than the whole horse serum.

Rabbit Antipneumococcus Type I Serum.—One subject, L. B., developed an immediate wheal associated with erythema and itching following the injection of 0.1 c.c. of rabbit serum. This subsided in an hour, and, as it was in every way characteristic of the immediate reaction which results in sensitive individuals from skin testing with the specific allergen, it was not considered a serum sickness reaction. L. B. has been working with rabbits, as well as guinea pigs and rats, for several years and now develops coryza in the presence of the latter two animals. J. C. was the only other one of the twelve subjects who reacted to the rabbit serum. Two hours after the injection wheals, erythema and itching developed, but the reaction was not so severe as the reaction to horse serum which occurred at the same time. By the following morning it had disappeared except for a slight residual erythema and itching. J. C. has also been working with rabbits for about two years. Two of the other ten, who failed to react to rabbit serum, have been associated with these animals extensively for several years, while six of the remaining have had contact with them recently. In summary, rabbit serum proved almost completely devoid of any ability to produce local serum sickness.

Sheep Antihemolytic Streptococcus Serum.—The results obtained following the intracutaneous injection of 0.1 c.c. of sheep serum were similar to those obtained with horse serum, although somewhat more frequent and severe. Reference to Table I will show that six of the twelve people inoculated developed wheals, erythema, and itching around the site of injection three to sixteen hours after injection. This reaction recurred for two to nine days. Fig. 1 illustrates one of M. J.'s reactions on the sixth day. Three other subjects did not react within the first twenty-four hours but had similar reactions later. G. H. developed wheals, erythema, and itching on the second and third days only. C. J. had intermittent attacks from the sixth to the twelfth day, while S. K. had recurrent wheals, erythema, and itching during the fourth, fifth, and sixth days after injection. The three remaining persons developed no outspoken local serum sickness, although A. O. complained of occasional attacks of itching during the sixth, seventh and eighth days. In summary, nine of the twelve subjects had a marked local serum sickness reaction after the injection of sheep serum, and one was troubled with itching at the site of injection on the sixth, seventh, and eighth days.

Bovine Diphtheria Antitoxin Serum.—All but one subject tested with this serum developed an immediate reddening which lasted one hour to a day and was apparently not related to any hypersensitive reaction. Eight of the ten individuals responded with a local serum sickness reaction, which was usually very mild. Two, S. K. and L. B., developed

a single wheal on the fifth and eighth days, respectively; while a third, G. H., had only two wheals on the second day and erythema and itching on the third day. Three other subjects reported more reactions, but each one was relatively slight and transitory so that the individual readily counted the number of wheals and gave the hour of the day when the reaction occurred, in contrast to the previous reactions of sheep and horse serum during which there was a multiplicity of wheals which came and went more or less continuously. The seventh and eighth subjects, M. J. and B. S., reacted for several days in a manner similar to that for horse and sheep serum, although the crops of wheals were not so large nor so persistent. Bovine serum, therefore, occupied a position intermediate between horse and sheep serum, on the one hand, and rabbit serum, on the other hand, in its ability to produce local serum sickness.

Bovine Tetanus Antitoxin Globulin.—All twelve persons injected with the cattle globulin developed an area of erythema 3 cm. to 8 cm. in diameter, which was slightly painful to pressure for one to two days. This reaction developed in a few minutes after injection and was quite uniform in all the subjects and apparently was unrelated to any type of allergic response. Three of the twelve subjects developed a delayed, very transitory and mild local serum sickness. G. H. had one wheal about 3 cm. in diameter which developed in the site of injection on the sixth day and lasted for one-half hour, while the associated itching persisted for another half hour. B. S. had a similar reaction on the seventh day. On the sixth day F. O. had a large area of erythema about 10 cm. in diameter which was associated with severe itching. Although no wheal developed, the erythema and itching were so pronounced that this also was considered a mild serum sickness reaction. The other nine subjects remained symptomless. The bovine globulin, therefore, elicited only a very mild transitory local serum sickness in three of the twelve persons tested.

DISCUSSION

The intracutaneous injection into the forearm of human subjects of 0.1 c.c. of undiluted sheep or horse serum obtained from hyperimmunized animals was effective in producing severe and protracted bouts of local urticaria associated with itching and erythema. Bovine serum, on the other hand, produced briefer and milder attacks, while horse globulin and bovine globulin were still less active in the order named. Rabbit serum was practically inactive in producing local serum sickness (see Chart 1).

There was no correlation between the previous parenteral administration of horse serum and the development of local serum sickness

to this antigen. Reference to Table I shows that S. K., although he had received horse antitoxin years previously, failed to react at any time following the injection of horse serum. This was not due to any constitutional lack of reactivity, since the sheep serum did provoke a local response. Of the remaining three who had a previous history of receiving horse serum parenterally, one, A. O., reacted to horse serum only; another, M. J., reacted both to horse and to sheep serum; and the third, G. H., reacted to horse, sheep, and cattle serum.

There was also no correlation between the occurrence of allergic diseases in this group of subjects and the ability to respond to the various serums with local serum sickness. It so happens that G. F. and D. K., the only two who failed to react to any of the serums, both have an allergic response to specific antigens. C. J., however, who also has an allergic background, had two of the severest local serum sickness reactions observed, while G. H. was one of the four who responded to three of the four serums.

A reaction to one serum does not imply that a given individual will respond with a local reaction to another serum which has the potentiality of eliciting a response in a large percentage of individuals. Thus A. O. reacted severely to horse serum but scarcely at all to sheep serum, while the reverse was the case with S. K. On the other hand, the minor reactions to rabbit and cattle serums occurred only in individuals who had reacted both to horse and to sheep serum.

It should be emphasized that these tests were made with only a single sample of serum or globulin from each species of animal tested. It has been observed that various lots of horse serum appear to differ in their serum sickness producing qualities. It may be that this method of local skin testing offers a means of distinguishing those serums from a given species of animal which produce particularly strong serum sickness reactions from those which do not. It, of course, remains to determine whether a correlation exists between the typical serum sickness which follows the parenteral injection of large quantities of a foreign serum and the local reaction to intracutaneous injection of small amounts of serum.

It was surprising to find that so many of the local reactions occurred after an incubation period of only a few hours. The cattle globulin was the only antigen which failed to produce a response for several days in the three who reacted to it. That these accelerated reactions should be considered as true local serum sickness might be questioned. Serum sickness following the parenteral injection of therapeutic serums usually occurs after an incubation of several days. Hunt found that in 804 cases of serum sickness following the injection of diphtheria antitoxin the largest number of reactions fell between the sixth and the

eleventh days. He reported 58 cases, however, within the first day. The local reactions which we obtained within the first twenty-four hours were identical in every respect with those which occurred after a longer incubation period. Furthermore, they usually lasted for several days, and we believe that they are to be interpreted as true local serum sickness.

The diversity of reaction which followed the injection of the 0.1 c.c. of serum could be explained in part on the basis of a slow diffusion of antigen into fresh tissue. Reactions which frequently recurred in the same area probably were due in part to a response to different antigenic fractions of the serum. The work of Hooker already referred to has explained the threefold repetition of a reaction to the intracutaneous injection of horse serum as due to the pseudoglobulin, euglobulin, and albumin fractions respectively.

The variation in the ability of serums from different animal species to elicit the local reactions remains unexplained. Both mutton and beef are common foods. If the local reactions were dependent on an acquired sensitization through contact with the foreign proteins, these two serums should be equally effective antigens. It is interesting in view of Davidsohn's evidence of the rôle which heterophile antibodies may play in serum sickness that horse serum and sheep serum, the two worst offenders in producing the local serum reaction, are the two serums of this group which contain Forssman antigen. The variations, however, in the response of a single individual to the different serums tested do not substantiate the theory that serum sickness is due only to one particular antigen.

SUMMARY

Ten or twelve normal adults were injected intracutaneously in the forearm with 0.1 c.c. of undiluted immune serum derived from four species of animals and with two globulin antibody solutions. Local serum sickness characterized by wheals, erythema, and itching occurred as follows:

1. Horse antipneumococcus Type I serum produced a severe, protracted local serum sickness in eight of the twelve individuals tested.

2. Sheep antistreptococcus hemolyticus serum produced a severe reaction in nine of the subjects and a slight reaction in one.

3. Rabbit antipneumococcus Type I serum produced a slight local reaction in only one individual.

4. Bovine diphtheria antitoxin serum produced a strong reaction in two and a moderate or slight reaction in six.

5. Equine tetanus antitoxin globulin elicited only two moderate and three slight reactions.

6. Bovine tetanus antitoxin globulin produced the mildest possible reaction in three individuals.

It is possible that the local serum sickness reaction may be helpful in studying the tendency of a therapeutic serum to produce serum sickness.

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PERTINENT FACTORS INFLUENCING COMPARATIVE SKIN TESTS ON THE ARM*

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IN 1920 Schloss[1] showed that the size of a positive reaction varies, depending upon its location on the arm. Several years later, Larsen, Paddock, and Alexander[2] confirmed this observation, reporting that the size of wheals produced by tests of like concentration and amount, on the forearm, decreases from the elbow downward to the wrist. More recently, Alexander and his associates[3] have shown a relative constant variation in the size of wheals on different parts of the body, namely the back, the thigh, and the forearm. This variation applies equally to wheals produced by histamine in normal persons and to those caused by specific skin tests in atopic individuals. The importance of these observations has been almost entirely overlooked by workers engaged in quantitative investigations involving the direct skin test. Their especial significance in procedures used in the comparison, by direct skin test, of two solutions differing but slightly in specific activity will be pointed out in this communication.

In a previous publication[4] I demonstrated that two dilutions of histamine, pollen extract, or *Ascaris lumbricoides* extract, differing in concentration by 25 per cent, can be distinguished from each other by appropriate skin tests. It was also shown that two dilutions of the same pollen extracts differing by only 15 per cent in specific activity could not be distinguished from each other by comparative skin tests. It is my purpose in this communication to discuss in detail the technic which should be employed in such comparative studies.

In an earlier investigation[5] of a different nature which also involved a comparison of wheal formation on the upper and lower portions of the arm, I noted that the higher site manifested an advantage in whealing power over the lower one. This advantage was frequently found to be great enough to counteract the effect of a slightly stronger stimulation at the lower site. Even when the reactivity of the upper site was deliberately impaired by previous trauma of various types, the advantage in position was frequently great enough to overcome this handicap, so that equal stimuli applied to this point and another, 4 inches lower, still produced larger wheals on the upper site. Hence, when comparing the effects of certain procedures on the whealing

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power of the skin, it was found necessary to alternate the positions of test and control injections and to average the results before a definite conclusion could be reached.

In comparing two extracts in order to determine which is the stronger, the importance of precision in technic cannot be overemphasized. The principles of intracutaneous testing as summarized by Walzer and Thommen[6] were strictly adhered to. I attempted to inject into the two test sites equal amounts of the two solutions being compared. With the testing equipment in ordinary use it is impossible to measure exactly the amount of material introduced, but in no instance was more than 0.01 c.c. of test material injected. Extreme care was taken to place the tests at the same depth in the skin and as superficially as possible.

I have found that in this type of work it is advisable to choose, for comparison, dilutions which induce slight (+) or moderate (++) wheals, since it is very difficult or even impossible to detect degrees of differences in very marked or very slight cutaneous reactions.

It has also been found necessary to compare the two solutions on more than one or two subjects because some skins do not reflect slight degrees of difference in stimuli. For this reason I have in each set of comparisons usually tested both arms of at least seven subjects and judged, from the results of these fourteen or more readings, which of the two solutions was the stronger. Only by using such a large number of comparisons can the relative strength of two solutions be correctly determined.

The solutions to be compared have, in every instance, been labelled by another worker in such a manner that I did not know which solution was the stronger. Fresh dilutions were prepared for each test day. The concentrated extracts from which the dilutions were made had been standardized on the total nitrogen basis as recommended by Coca.[7, 8]

Accurate reading of the reactions was found to be an extremely important factor in this investigation. Superficial examination of reactions frequently led to erroneous conclusions. Two wheals which appeared upon inspection to be equal were often found to be unequal on palpation. The readings of the reactions were therefore based not only on the apparent size of the wheal but also on the amount of edema present at the wheal site. It will be noticed by an examination of the tables that the larger wheals were not always accompanied by the greater amount of erythema; in fact, the reverse was quite often true. The latter consideration is important and should be kept clearly in mind by the worker who is making comparative skin tests.

In order to guard against any possible prejudice on the part of the investigator as a result of first impressions gained from the early

readings in a series, the following plan was adopted. When the test injections were made, a diagram was drawn of the arrangement of the tests injected in each subject. Later, when the reactions resulting from these tests were read, they were recorded elsewhere by means of another diagram. Since several patients were tested in succession, and since the arrangement of tests varied in each subject, I did not know, when reading each reaction, which solution had produced it. The two diagrams were not coordinated until the entire series was completed.

The results of tests performed with two ragweed solutions differing by 25 per cent in specific activity are shown in Table I. The experiment was carried out, according to the technic described above, on 14 subjects known to be specifically sensitive to ragweed pollen. The tests were injected in a vertical line from 4 inches to 5 inches apart on the upper arms of the subjects. On one arm of each subject the test with Solution A was placed above that with Solution B; on the other arm, the tests were injected in the reverse order. It will be noticed that, when the test with the weaker solution was placed uppermost on the arm, the stronger solution was correctly identified in only 7 of the 14 tests done. In other words, when the test with the weaker solution was uppermost on the arm, the stronger solution produced a larger reaction in only 50 per cent of the tests performed with it; but, when the test with the stronger solution was placed uppermost on the arm, the stronger solution was identified in 11.5* of 14 instances, or in 82 per cent of the tests. The average of these two results (50 per cent and 82 per cent) is 66 per cent, which represents the percentage of instances in which the stronger of two solutions differing by 25 per cent in concentration was correctly identified.

These same reactions were read independently by another worker who was not present when the test injections were made and who did not know in what manner the solutions had been labelled. This worker was able to identify the stronger solution in 54 per cent of cases when the test with the weaker solution had been uppermost on the arm and in 71 per cent of cases when the tests had been injected in the reverse order. The average of these two results is 62.5 per cent, which represents the percentage of cases in which the stronger solution elicited the larger reaction. The slight discrepancy between the percentages obtained by this worker and those which I obtained in this and subsequent experiments was due to the differences in interpretation which are to be expected in the reading of reactions differing but slightly in their respective sizes.

*When the two reactions were considered equal in size, the two solutions were each credited with half a count. This explains the fractions found in the figures quoted.

TABLE I

RESULTS OF COMPARATIVE TESTS WITH SOLUTIONS VARYING IN STRENGTH PLACED 4 INCHES APART VERTICALLY ON THE UPPER ARM

SOL. A = 2 R 0.001 MG. N PER C.C. — SOL. A UPPERMOST						SOL. B = 2 R 0.00125 MG. N PER C.C. — SOL. B UPPERMOST					
PATIENT	ARM	TEST SOLUTION	WHEAL	ERYTH.	SOLUTION GIVING LARGER REACTION	PATIENT	ARM	TEST SOLUTION	WHEAL	ERYTH.	SOLUTION GIVING LARGER REACTION
D. L.	R	A	+	±		D. L.	L	B	+±	+	B
		B	++	+	B			A	+	0±	
F. F.	L	A	++±	+±	A	F. F.	R	B	+++	++	B
		B	++	+				A	++±	+±	
A. M.	L	A	+±	+		A. M.	R	B	++±	+±	B
		B	++±	±	B			A	+±	+	
S. K.	R	A	+	+		S. K.	L	B	+±	±	B
		B	+±	±	B			A	+	+	
E. R.	R	A	+±	±	A	E. R.	L	B	++	±	B
		B	+	0±				A	+±	±	
B. B.	L	A	+	+		B. B.	R	B	+	+±	B
		B	+±	+	B			A	+	±	
N. K.	R	A	+	+		N. K.	L	B	+±	+	B
		B	+±	0±	B			A	+	±	
I. K.	L	A	++±	++	A	I. K.	R	B	+±	±	A
		B	++	+				A	++	+	
H. F.	R	A	++	+	A	H. F.	L	B	+±	±	
		B	+±	±				A	+±	±	Equal*
He. F.	R	A	+	±		He. F.	L	B	+	+	A
		B	+±	+	B			A	+±	+±	
E. G.	L	A	++±	+±	A	E. G.	R	B	++±	++	B
		B	++	+				A	++	+	
K. O.	R	A	+	+		K. O.	L	B	+±	+	B
		B	+±	+	B			A	+	+	
J. M.	L	A	++	+	A	J. M.	R	B	++	+	B
		B	+±	±				A	+±	+	
M. L.	L	A	++±	0 0±	A	M. L.	R	B	++±	0±	B
		B	++	0 0±				A	++	0±	
A > B 7 readings B > A 7 readings			50% correct			A > B 2.5* readings B > A 11.5* readings			82% correct		

Average of correct readings for both groups 66 per cent.

*Whenever the two solutions elicited equal reactions, the numerical value 0.5 was given to A and B.

In another series of tests which I made on 20 patients in the same manner, the stronger solution produced a larger reaction in 64 per cent of the comparative tests done. The results of these two series of tests indicate that the stronger of two solutions was correctly identified in two out of every three comparisons. I, therefore, believe that two solutions differing by 25 per cent in specific activity can be differentiated from each other by direct skin test when the two tests are placed vertically about 4 inches apart on the arm and when their relative positions are alternately reversed.

The next study was made to determine the results of comparative tests with two solutions of ragweed extract differing in specific activity by 25 per cent when the tests were placed vertically on the arm approximately 2 inches apart. The findings are shown in Table II. The stronger solution was identified in only 29.2 per cent of the cases tested when the test with the weaker solution was placed uppermost on the arm, and in 66.6 per cent of cases when the tests were placed in the reverse order. The average of these percentages is 47.9 per cent, i.e., the stronger solution produced the larger reaction in only 47.9 per cent of the cases tested, while the weaker solution produced a larger reaction in 52.1 per cent of cases.

Independent readings by another worker revealed an identification of the stronger solution in 50 per cent of cases when the test with the weaker solutions was uppermost, and in 66.6 per cent of cases when the relative positions of the tests with the two solutions were reversed. The average, 58.3 per cent, represents the percentage of cases in this series in which the second worker was able to identify the stronger solution.

In still another series of 20 patients tested in the same manner, I found that larger reactions were produced by the stronger solution in only 55.5 per cent of the cases tested. The corresponding percentage obtained from the readings of another worker was only 47.2 per cent. Hence, my average for the correct identification of the extracts in the two series (47.9 per cent and 55.5 per cent) was 51.7 per cent; the other worker's was 52.7 per cent.

These results indicate that under the conditions of this experiment, the stronger solution produced the larger reaction in about the same number of instances as did the weaker solution. It must, therefore, be concluded that two solutions differing in specific activity by 25 per cent cannot be differentiated from each other by direct skin test when the tests are placed vertically on the upper arm approximately two inches apart. In the preceding experiment, however, such a differentiation could be made when the tests were introduced four inches apart. Hence, the proximity of the tests to each other in the vertical plane was a decisive factor in the success or failure of the technic.

TABLE II

RESULTS OF COMPARATIVE TESTS WITH SOLUTIONS VARYING 25 PER CENT IN STRENGTH PLACED 2 INCHES APART VERTICALLY ON THE UPPER ARM

SOL. Y = 2 R 0.001 MG. N PER C.C. — SOL. Y UPPERMOST						SOL. X = 2 R 0.00125 MG. N PER C.C. — SOL. X UPPERMOST					
PATIENT	ARM	TEST SOLUTION	WHEAL	ERYTH.	SOLUTION GIVING LARGER REACTION	PATIENT	ARM	TEST SOLUTION	WHEAL	ERYTH.	SOLUTION GIVING LARGER REACTION
C. H.	R	Y	+±	0±	Y	C. H.	L	X	+±	±	X
		X	+	0 0±				Y	+	0±	
V. O.	L	Y	++±	0±		V. O.	R	X	+++	++	X
		X	++±	0±	Equal			Y	++±	±	
M. S.	L	Y	+±	±		M. S.	R	X	+±	±	X
		X	+±	±	Equal			Y	+	±	
J. T.	R	Y	++	+±		J. T.	L	X	++	±	X
		X	++±	+	X			Y	+±	±	
M. B.	L	Y	+±	±	Y	M. B.	R	X	+	0±	Y
		X	+	0±				Y	+±	0±	
G. G.	L	Y	+±	+	Y	G. G.	R	X	+	+	
		X	+	±				Y	+±	+±	Y
G. S.	R	Y	+	0±	Y	G. S.	L	X	+±	0±	X
		X	±	0 0±				Y	+	0	
L. T.	R	Y	+±	+±	Y	L. T.	L	X	+±	±	X
		X	+	+				Y	+	±	
R. S.	L	Y	+±	±	Y	R. S.	R	X	+	±	X
		X	+	±				Y	±	±	
Y. A.	R	Y	+±	±	Y	Y. A.	L	X	+	0 0±	
		X	+	0 0±				Y	+±	±	Y
M. S.	L	Y	+±	0 0±	Equal	M. S.	R	X	+±	0±	
		X	+±	0 0±				Y	++	0±	Y
S. P.	L	Y	±	0 0±		S. P.	R	X	+±	±	X
		X	+	0 0±	X			Y	+	0±	

Y > X 8.5 readings } 29.2% correct
X > Y 3.5 readings }

X > Y 8 } 66.6% correct
Y > X 4 }

Average of correct readings for both groups 47.9 per cent.

The next point to determine was the influence of tests upon each other when they were introduced horizontally on the arm. In Table III are recorded the results of 15 comparative tests performed on 8 patients with dilutions of ragweed pollen extract which, in this experiment, differed in specific activity by only 20 per cent. These tests were introduced approximately 1.5 inches distant from one another in a horizontal line on the arm. In 7 of these 15 comparative tests, the test with the stronger solution was placed medially, and in the remainder laterally. A larger reaction was obtained with the stronger solution in 81.2 per cent of the former cases and in 71.4 per cent of the latter. The average of these results, 76.3 per cent, represents the percentage of cases in which the stronger solution was identified. In another series of 14 comparative tests, the stronger solution produced the larger reaction in 57.1 per cent of cases when the test with the stronger solution was placed laterally and in 100 per cent of cases when it was placed medially. The average of these two results is 78.5 per cent, which represents the percentage of cases in which the stronger solution was correctly identified. The readings of these same reactions by another worker showed average percentages differing but slightly from those which I obtained.

If the percentages of results in these two series of cases be averaged with respect to the relative positions of the tests, we find that the stronger solution was correctly identified in 70 per cent of the cases when the test with this solution was placed laterally and in 85.6 per cent of cases when it was placed medially. This seems to indicate a slight advantage of the medial position on the upper arm. For this reason, it is advisable, even when comparative tests are made in the horizontal plane, to alternate their relative positions on the arm.

Summarizing the results of the experiments which are charted in Table III, it would seem that when the tests with two solutions differing by 20 per cent in specific activity are placed horizontally on the upper arm approximately 1.5 inches apart, the stronger solution can be correctly identified in approximately three out of every four comparative tests made.

As different skins vary considerably in the degree of their response to the same stimulus, there does not seem at present to be any suitable standard or unit in terms of which their comparative whealing powers can be measured. However, for the purpose of arriving at some estimate of the extent of the superiority in whealing power which the upper portion of the arm manifests over the lower, I performed 63 comparative tests on 40 atopic patients using the same pollen extract in both positions. These tests were placed vertically on the arm approximately 4 inches apart. In 67.85 per cent of the comparative tests done, the resulting wheals on the *upper portion* of the arm were

TABLE III

RESULTS OF COMPARATIVE TESTS WITH SOLUTIONS VARYING 20 PER CENT IN STRENGTH PLACED 1.5 INCHES APART HORIZONTALLY ON THE UPPER ARM

SOL. N = 2 R 0.001 MG. N PER C.C.						SOL. M = 2 R 0.00125 MG. N PER C.C.					
SOL. N MEDIALLY						SOL. M MEDIALLY					
PATIENT	ARM	TEST SOLUTION	WHEAL	ERYTH.	SOLUTION GIVING LARGER REACTION	PATIENT	ARM	TEST SOLUTION	WHEAL	ERYTH.	SOLUTION GIVING LARGER REACTION
F. B.	R	N	+ ±	+		M. P.	R	N	+ +	±	N
		M	+ +	+	M			M	+ ±	±	
F. B.	L	N	+ ±	±		M. P.	L	N	+ ±	0	
		M	+ +	±	M			M	+ +	±	M
S. M.	R	N	+ ±	±		B. D.	L	N	+ +	0 ±	N
		M	+ +	0 0 ±	M			M	+ ±	0 0 ±	
S. M.	L	N	+ ±	±		B. D.	R	N	+ +	+	
		M	+ +	±	M			M	+ + ±	+	M
S. K.	R	N	+ ±	+ ±	N	F. S.	R	N	+ +	+	
		M	+	+				M	+ + ±	+ ±	M
S. K.	L	N	+	+		F. S.	L	N	+ ±	0 ±	
		M	+ ±	+	M			M	+ + ±	±	M
E. G.	R	N	+ ±	±		J. F.	L	N	+	+	
		M	+ + ±	+	M			M	+ ±	±	M
E. G.	L	N	+ + ±	+							
		M	+ + ±	+	Equal						

M > N 6.5 readings, N > M 1.5 readings } 81.2% correct

M > N 5 readings, N > M 2 readings } 71.4% correct

Average of correct readings for both groups 76.3 per cent.

larger than those on the lower. It is obvious that this percentage closely approximates the average obtained when the same experiment was carried out with pollen solutions differing by 25 per cent in specific activity and when the relative positions of the tests were reversed in half of the cases tested. (Table I.) This would seem to indicate that a distance of 4 inches on the arm represents a difference of 25 per cent in the strength of the stimulus employed in this experiment. Further evidence in favor of this assumption may be found in Table I. Here it will be noticed that when two pollen solutions varying 25 per cent in strength are introduced 4 inches apart in a vertical line on the arm with the stronger solution in the lower position (and therefore at a disadvantage), the latter can be recognized as the stronger solution in exactly 50 per cent of the comparative tests. In the other 50 per cent of cases, the weaker extract (in the advantageous position, i.e., 4 inches higher up on the arm) gives the larger reaction. I present this evidence not as an exact measure of the advantage in whealing power on the upper portion of the arm over that on the lower but as an indirect means for roughly estimating that advantage which has been found to be present.

The above statements do not apply to tests performed in the same manner with the nonspecific excitant, ergamine acid phosphate (histamine). Sixty-nine comparative tests were performed on 47 atopic patients, using histamine 1:5,000 in both positions. Both these tests were placed in a vertical line 4 inches apart. In 61.6 per cent of the tests performed the resulting wheal on the *lower portion* of the arm was larger. The explanation for this finding is not at present known. It is possible that my recently reported[5] work on the restimulation of previously tested wheal sites may shed some light on the subject. In the latter experiments results were obtained which strongly suggest that the mechanisms of specific (reaginic) and nonspecific (histamine) wheal formation in atopic individuals are not the same.

In experiments of this nature there is considerable error due to variations in technic beyond the control of the worker and to mistakes in the reading and interpretation of reactions. Hence, one should not lay too much stress on final results which show only slight differences in the percentages obtained. I, however, consider the findings significant when consistent differences are recognized in at least two-thirds of a relatively large number of cases and have therefore adopted 66 per cent as the minimal percentage of consistent results required before any conclusions can be drawn regarding the relative strengths of the two solutions. It should be clearly understood that this 66 per cent is valid only when it represents the average of results in a series of tests in which the relative positions of the tests with the two solutions are alternated. It would be invalid if it represented the results

obtained with one solution always in the upper position and therefore at an advantage. For example, in my previously reported studies, the stronger of two solutions differing from each other by 15 per cent gives a larger reaction in 64 per cent of cases when it is in the position of advantage, but in only 34 per cent of cases when in the reverse position. In the latter instance, the 64 per cent is obviously an invalid result.[4]

If, when testing with pollen extracts, the one reaction is found to be larger than the other in a high percentage of cases, reversing the relative positions of the two tests does not sufficiently influence the results to change the original impression regarding the relative strength of the two solutions. For example, it has been found that when the stronger pollen solution in the upper position (at an advantage) gives larger wheals in 80 per cent or more of the comparative tests, it can be definitely identified as the stronger extract without further testing. A reversal of position in such an instance results in a larger wheal by the stronger pollen extract in the inferior position (at a disadvantage) in 50 per cent of cases (Table I). The average of the two is 66 per cent thus making the result valid. One can therefore consider a finding of 80 per cent or higher to be irreversible by a change of position if the proper technical procedures as outlined above are followed. The latter statement is also applicable to comparative tests with histamine.

I took this into consideration in the experiments reported in a previous communication.[4] In the comparative tests with two pollen solutions differing from each other by 25 per cent, the stronger solution was identified in 84.4 per cent of the first comparative tests when the test with this extract was in the upper position. This high figure made further study using the reversed position unnecessary. In like manner, the stronger solution was immediately identified in 87.5 per cent of the first tests performed with extracts of *Ascaris lumbricoides* differing by 25 per cent in activity and in 81.7 per cent of the first comparative tests with solutions of histamine differing 25 per cent in strength. On the other hand, in studying two ragweed solutions differing by 15 per cent in specific activity, the stronger extract was identified in only 64 per cent of cases when the test with it was in the position of advantage. A reversal of the relative positions of the tests with these two extracts was therefore necessary. This resulted in an identification of the stronger solution in only 34.4 per cent of cases. The average of these two results was only 49.2 per cent, and I therefore concluded that two pollen extracts differing by 15 per cent in specific activity could not be differentiated from each other by the direct skin test.

DISCUSSION

The above experiments have shown that, in comparing two solutions by means of the direct intracutaneous skin test, the slight difference in the specific activity of the two pollen solutions is most easily detected by placing the tests in a horizontal line about 1.5 inches apart on the upper arm.

It has likewise been demonstrated that a 25 per cent difference in the specific activity of two solutions can be detected when the tests are placed in a vertical line on the upper arm about 4 inches distant from one another. On the other hand, it is impossible to detect this same difference when the tests are placed in a vertical line 2 inches apart.

For the reasons stated previously it is advisable, when comparing two solutions differing but slightly in specific activity in order to determine which of the two is stronger, to reverse the positions of the two tests in about one-half of the cases tested. This latter procedure is absolutely essential when the tests are placed vertically on the arm. It can be omitted only when the extract in one position induces consistently larger reactions than the other in more than 80 per cent of cases.

The fact that these differences in reactions could be detected by a second worker, who had not been present when the tests were injected, indicates that this technic for comparing two solutions is one that can be employed by any worker familiar with the reading of reactions resulting from intracutaneous tests.

A point of clinical importance in the daily routine of cutaneous testing is suggested by the above experiments. It has been pointed out that tests performed in a vertical plane on the arm influence each other to a far greater degree than those placed in a horizontal plane. Under the circumstances it seems advisable in performing routine cutaneous tests to insert them in rows across the arm rather than in rows up and down the arm. It has been demonstrated that proximity of the tests to each other is also an important consideration and that tests should be spaced as far as possible from each other. (This latter factor does not pertain to comparative tests but to direct tests in the diagnosis of hypersensitiveness.) These points will be the subject of a future communication.

SUMMARY

1. The technic which has been found most useful for comparing, by direct skin tests, two solutions differing but slightly in specific activity is described.

2. A dilution of pollen extract which is 25 per cent stronger than another dilution of the same extract can be identified in 66 per cent

of the comparative tests performed with the two solutions when the tests are placed vertically on the arm approximately 4 inches apart and when the relative positions of the tests with the two solutions are alternately reversed in one-half of the cases tested.

3. When these same extracts are compared by placing the tests performed with them vertically on the arm only 2 inches apart, the stronger solution elicits the larger reaction in only 47.9 per cent of the cases tested. The two solutions, therefore, cannot be differentiated from each other by this technic.

4. A dilution of pollen extract differing in specific activity by 20 per cent from another dilution of the same extract can be identified in 76.3 per cent of the cases tested when the tests with the two solutions are placed horizontally on the arm approximately 1.5 inches apart and when the relative positions of the tests are alternately reversed in one-half of the cases in the series.

5. The upper position on the arm shows advantage in whealing response to tests with pollen extracts over the lower position and the medial over the lateral, but to a lesser degree in the latter instance than in the former.

6. In these same atopic subjects, the lower position on the arm shows advantage in whealing response to tests with histamine over the upper position.

7. Tests inserted in rows vertically on the arm influence each other more than those inserted in horizontal rows. This point should be taken into consideration in routine cutaneous testing for hypersensitivity.

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DISCUSSION

DR. HUBER.—The utility of this paper, I think, was not emphasized as it should be. We frequently are called on to change from one extract to another in treatment, and, if skin tests mean anything at all, one has here a method of making a comparison of the strength or the differences in strength of these extracts. Dr. Harsh and I have done quite a little work on the comparison of extracts, and we have found the scratch method to be much more accurate than the intradermal method of testing.

DR. RAPPAPORT.—My experience has been very similar to Dr. Huber's in attempting to compare for experimental work the activity of vari-

ous solutions. I found it impossible to make any degree of comparison by using the intracutaneous method. If one compares the activity of two solutions, one of them twice as strong or even five times as strong as the other, no definite conclusion can be arrived at regarding the relative potency of the materials by the intracutaneous method.

I have tried for several years to perfect my own technic in this observation because it is an important problem to me. I might be able to say that the one which is five times as strong is a stronger solution, but I could not possibly arrive at anywhere near a comparison of the two solutions.

With the scratch method I have used an instrument which gives the same size scratch and the same depth of scratch. With this I have been able to make closer comparisons of the relative potency of different materials provided they are strong enough to produce a reaction with the scratch test. I do believe that the scratch method for comparing the different strengths of solutions is superior to the intracutaneous method.

DR. LOUIS TUFT.—I should like to ask Miss Bowman whether in carrying out these tests she used any subjects that were receiving injections of pollen extract in the course of treatment. The reason I ask is that during the experiments which we shall report a little later in the morning, we found in doing skin tests in those areas in which injections of pollen extract had recently been given, a very much smaller reaction was obtained. These areas seem to be somewhat refractive, possibly as a result of a temporary desensitization. I wondered whether Miss Bowman has had any similar experiences.

DR. STERNBERG.—About five years ago I collected some twenty-five patients who gave equal and maximal reactions to ascending concentrations of ragweed pollen extracts. That is, these patients gave marked and equal local skin reactions with extracts containing 0.0001, 0.001, 0.01 and 0.1 mg. of nitrogen.

Four of these patients, who also suffered from early hay fever, were tested with timothy extract and gave the usual skin reactions to the various concentrations.

My explanation at that time was that the reagin content in these patients was limited to ragweed, and apparently not to timothy.

Some of these patients were treated in our clinic in previous years with poor results. Because of the fact that they gave marked reactions to the 0.0001 mg. extract they had been classed and treated as Class A patients. Placing them in Class C we were able to give them more relief. These patients seem to stand the treatment much better than the usual ones. They appear refractory to a certain degree. They rarely complain of a local reaction, and in the small number studied no constitutional reactions were observed.

This observation may explain some of the tests Miss Bowman has demonstrated.

DR. LEO H. CRIEP.—Would this work attempt to explain the reason for a weaker solution of pollen giving a stronger skin reaction? Is it a difference in technic or a difference in location, granting that the amount injected is exactly the same?

DR. MATTHEW WALZER.—There is one aspect of this paper that has caused no discussion. I wish it had because I think it is the most important aspect of the paper. I refer to the difference in results obtained on testing with ragweed and histamine when these tests are placed up and down the arm. You noticed that when two tests with the same solution of histamine were put in the upper and lower parts of the arm 4 inches apart, the lower histamine test gave a larger reaction in the majority of cases. The reverse was true when the same thing was done with ragweed. There must be some reason for this.

We have been told repeatedly that the mechanism of the immunologic reagin reaction and that of the histamine reaction are the same, namely, that as a result of the reagin-allergen reaction there is a liberation of some histamine or histamine-like substance which causes the wheal. That may be so, as far as the formation of the immediate wheal is concerned, but I think we ought to realize that the reagin reaction is in many respects entirely different from the histamine reaction.

This is the second piece of work we have had to prove that point. I should like to show the first bit of evidence. Table I is from a paper presented by Miss Bowman and myself, before the Society of Immunologists two months ago. If you initially test a skin site on atopic patients with histamine and repeat the same test on the same site one week later, and compare this reaction with that obtained on a previously untested site, it will be found that the control site gives a larger reaction in 80 per cent of the cases. This indicates that there is refractoriness to whealing at the site previously tested with histamine.

TABLE I

RESPONSE OF WHEAL SITES ON ATOPIC SUBJECTS TO RESTIMULATION AFTER SEVEN-DAY INTERVAL

EXCITANTS			WHEAL		ERYTHEMA	
			LARGER ON		LARGER ON	
FIRST	SECOND	NUMBER OF TESTS	RETESTED SITE	CONTROL SITE	RETESTED SITE	CONTROL SITE
Histamine 1:1,000	Histamine 1:5,000	48	20.5%	79.5%	14.7%	85.3%
Histamine 1:1,000	Pollen extract	90	52.5%	47.5%	33.0%	67.0%
Pollen extract	Histamine 1:5,000	45	89.8%	10.2%	16.7%	83.3%
Pollen extract	Pollen extract	77	77.0%	23.0%	17.6%	82.4%

Pollen extracts: timothy and ragweed.
Test injection: 0.01 c.c.
Interval between tests=seven days.

If a site is tested with pollen extract and retested one week later with histamine and the result is compared with that obtained on a normal site, one obtains a different result. In 90 per cent of cases the wheal on the retested site is larger than that on the normal site.

When one tests a site with pollen and retests it with pollen one week later, one obtains larger reactions on the retested site than on control sites in 77 per cent of cases. If one tests with histamine first and retests with pollen, the results on retested sites and controls are alike.

This evidence seems to indicate that the reagin reaction produces effects on the vessels of the skin which are different from those produced by histamine.

I believe Miss Bowman's findings showing different reactivities on various parts of the skin to reagin and histamine tests are further proof that histamine reactions and specific reagin reactions are not identical mechanisms.

Miss Bowman.—I am not well qualified to state what can be done by the scratch method. I have tried it and I cannot detect 25 per cent differences with it. That, however, may be my fault. I have had more experience with the intradermal than with the scratch technic.

In answer to Dr. Tuft, I have had the same experience he has had. I prefer to do these tests, as all these were done, on patients who have not received any treatment since the previous fall. I test them in the spring. I also use new patients coming into the clinic who have never received treatment.

In answer to Dr. Criep's question about the weaker solution, I am unable to answer that. I get it quite often myself. I think sometimes location on the arm is the factor involved and sometimes it is due to faulty technic. I think the depth of the injection has a great deal to do with the result obtained. The more superficially the test is placed, the more consistent the result obtained.

TRICHOPHYTIN AND MONILIA EXTRACTS IN ALLERGIC DERMATOSES

Report of Cases*

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A CERTAIN number of cases of eczematous dermatoses appear to be due to a hypersensitivity to ringworm organisms or to their products. Sulzberger and Wise[1] reported a series of these cases, basing their diagnosis on the reactions to skin tests and the results of treatment with trichophytin and oidiomycin† extracts. Numerous other references to such cases are found in the literature.[2-9] These cases exhibit dermatophytids, which are the inflammatory eczematous reactions to a primary skin focus of fungus infection, that is, the dermatophytosis.

Our work has been done along the lines laid down by Sulzberger and Wise, using as testing solutions principally the dermatomycins‡ prepared by Lederle. It was begun in an attempt to differentiate and treat some of the eczematous reactions found in allergic patients. Certain of these patients gave definite skin reactions to foods and inhalants, yet the skin lesions remained unchanged when these positive allergens were eliminated from the patient's environment although the general health and some symptoms of the patient might improve. One or two of these patients gave a history of long standing ringworm infection of the feet and inguinal regions antedating the onset of the dermatosis. This caused us to look for sensitivity to fungus infection, as it seemed probable that some allergic patients would be hypersensitive to a fungus infection as widespread as ringworm and not unlikely that in some of these cases the manifestation of the allergy would be a dermatosis. Sulzberger and Wise's article on "Ringworm and Trichophytin" had already been published, and other reports of trichophytids were numerous.[6-9] Hypersensitivity to bacteria with resulting allergic manifestations had become an accepted concept. Our next step was to find out how many of these resistant and possibly allergic dermatoses would improve under attempted desensitization with extracts of ringworm organisms. The cases reported are selected from

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†Oidiomycin is a term used in this paper to designate extracts of monilia.

‡The term "dermatomycins" has been suggested by Sulzberger as a convenient designation of the group of substances that are apparently responsible for the allergic manifestations of the dermatomycoses.

those seen in the allergy clinic at the Vanderbilt University Hospital and from the private practice of the author. Some were referred as cases of dermatitis probably due to allergy and others occurred in patients with other allergic manifestations.

METHODS

At first cases of dermatosis of doubtful origin were selected and studied. Later tests with the dermatomycin extracts were given routinely to all the eczematous patients in my practice as well as to patients with certain other allergic manifestations. These patients were questioned for a history of personal and family allergy, were often skin tested for the common allergens, were questioned and examined for evidence of ringworm infection, and were skin tested with extracts of two dermatomycins. These were the so-called trichophytin and oidiomycin of Lederle and, in a few cases, the trichophytin prepared by Metz. All tests were given intradermally only, in doses of 0.1 c.c. or 0.05 c.c. of 1:30 dilution of the trichophytin of Lederle, 1:50 dilution of the trichophytin of Metz, and a 1:100 dilution of the oidiomycin of Lederle. In some acute or severe cases, however, after experience had taught us that these doses frequently caused unpleasant exacerbations of the skin lesions with itching, initial doses were given in higher dilutions. Treatment was begun with a dose that gave a mild skin reaction with general improvement of the lesions and then gradually increased in amount at regular intervals as indicated by the strength of the reaction.

The local skin reactions to these injections were of two types: one, the delayed reaction reaching its height in twenty-four or forty-eight hours, comparable to the tuberculin reaction; and the other, the immediate or wheal reaction which is often accompanied by itching. The trichophytin extract more frequently gave the immediate reaction while the oidiomycin, as a rule, gave a delayed reaction which usually reached its maximum on the second day. In some cases, however, both gave the immediate reaction. Since infection with ringworm is widespread and the test is probably specific for infection in much the same way as is the tuberculin test, a positive reaction was judged to mean only evidence of past infection and not that the dermatosis for which the patient sought relief was due to hypersensitivity to the fungus infection. If, however, as was frequently the case, the tests were followed by an improvement of the skin lesions together with a moderate local reaction, or by an exacerbation of the skin lesions with a coincident excessive local reaction to the tests, then it was considered that sensitivity to the fungus infection was a factor in the dermatosis.

In the cases in which the trichophytin prepared by Metz was used with the trichophytin and oidiomycin prepared by Lederle the two

trichophytin extracts gave identical reactions when used in comparable dilutions, but the reactions were not parallel with those of the oidiomycin extracts. This led us to conclude that the oidiomycin extract, which is the extract of the monilia group of fungi, and the trichophytin extract are distinct immunologically and that both types of extracts must be used in skin testing. In a few cases in which trichophytin gave negative skin tests oidiomycin was used alone with good therapeutic results.

RESULTS

In Tables I and II are tabulated 33 cases. They do not include all patients tested with the trichophytin extracts but include as far as possible all the cases in which the skin tests with the trichophytin extracts showed not only a positive reaction but also a definite alteration of the clinical condition corresponding in time with the height of the local reaction. Some patients were necessarily excluded because they did not return for observation or treatment with regularity.

The 33 patients are arranged in two groups. In the first group (patients 1 to 12) no treatment was given except injections of trichophytin and oidiomycin extracts (Table I); in the second group (patients 13 to 33 inclusive) are the cases in which other methods were also employed (Table II); that is, exclusion of or treatment for other positive allergens along with the fungus extract.

Of the 12 patients in Group I, nine patients were completely cured, one was definitely improved and two were unimproved. Four of these patients (Nos. 2, 6, 7, and 8) were particularly noteworthy. In all of these, skin lesions in a severe form had been present for twelve years or longer without remissions in spite of x-ray therapy and other treatments, and all cleared up promptly under treatment with the fungus extracts. Patient 1 was improved but did not return after the fifth treatment, at which time improvement was marked. Patient 5 was unimproved and was made much worse by the sixth injection, at which time the skin lesions spread from the feet and ankles all over her body. After this she was afraid to try even a minute treatment dose. Patient 11 was unimproved, has a dermatitis of the hands in winter only, and is still under treatment.

Of the 21 patients in Group II, 12 were completely cured, 5 were improved, 3 were unimproved, and one was doubtfully improved.

Although in the second group of patients it was impossible to evaluate accurately in each case the effect of the various parts of the treatment, yet the definite relation of the local skin reaction to the clinical course of the disease definitely marked each case to the clinician as a fungus sensitivity dermatosis. In each case a moderately positive skin reaction reaching its height the second day after the injec-

tion was coincident with improvement of the clinical condition; a very marked local reaction at the site of injection was at its height usually accompanied by an exacerbation of the clinical condition and was often accompanied by malaise, some nausea, and headache. A treatment giving no local reaction was attended by no change in the clinical condition.

It is a matter of interest that two of the patients presented showed other allergic manifestations which appeared to be due to sensitivity to the oidiomycin organisms. Patient 2 of Group I had had a persistent perennial rhinitis for fourteen years with relief for the first time following injections of oidiomycin. Excessive local skin reactions from the treatment in this case were accompanied by general reactions consisting of severe attacks of sneezing and rhinitis. Patient 18 showed a persistent hydrarthrosis of the left knee joint of two years' duration which cleared up steadily after administration of oidiomycin for dermatitis of the hands. This joint had been studied and treated by various methods (including x-ray, aspiration, injection of joint fluid into a guinea pig, the application of heat and fixation) for several months previously with no decisive or favorable results. Patient 29 responded to an overdose of fungus extract with a large local reaction and a severe attack of vasomotor rhinitis.

General reactions consisting of headache, nausea, weakness, and general malaise were not unusual following doses of the fungus extract, which, as judged by the local reaction, were excessive.

Patient 16 gave general reactions to the fungus injections severe enough to cause her to remain in bed for two days. She suffered severe headaches and feelings of nausea and faintness the second day after the second treatment, which consisted of 0.05 c.c. of each extract. Although the dose was progressively reduced until the fourth treatment was only a fifth of the original strength, it produced a like reaction. After the fifteenth treatment the hands were definitely improved, but the patient still had headache the second day. This patient ceased coming after the nineteenth treatment because her hands were about well and she dreaded the general reactions. Her final dose was 0.18 c.c. of trichophytin 1:300 and oidiomycin 1:1,000. Patient 32 and patient 25 suffered particularly annoying general reactions after several treatments.

In the tables several types of observations are presented. Under "localization and extent" are noted the location, the size, and the severity of the lesions. Under "duration" is indicated the duration of the lesions. Under "procedure" is indicated the form of treatment employed: whether injections of trichophytin extracts alone, or whether treatment included injections of other allergens. Under

TABLE I

GROUP I

NAME	LOCALIZATION AND EXTENT	DURATION	PROCEDURE	RESULTS
Patient 1 Male, white Age 29	Irritated scaly lesions, hands, nails, feet and face.	7 months. Ringworm of feet several summers.	Injections of trichophytin and oidiomycin.	Improved second day after test dose and after successive treatments. Returned for only 5 injections.
Patient 2 Male, white Age 44	Eruption, reddish, itching rash both forearms, legs below knees, and tops of feet.	15 years. No remissions.	Injections of oidiomycin.	Cured after 22 treatments. No recurrence in 6 months.
Patient 3 Female, white Age 30	Small spot of eczema on left arm and on back of neck.	Back of neck at times for 17 years. Arm several years constant.	Injections of trichophytin and oidiomycin.	Skin lesions cleared. No recurrence three months later.
Patient 4 Female, white Age 34	Eczema of skin of hands between and along sides of fingers.	----------	Injections of trichophytin and oidiomycin.	Cured after two treatments. No recurrence one year later.
Patient 5 Female, white Age 51	Eczema of feet and ankles. Covered whole surface. Red, weeping, itching, crusted.	5 years. No remission. Some improvement at times.	Injections of trichophytin and oidiomycin.	Definite improvement after 2nd and 3rd injections. Exacerbations after 4th, 5th and 6th injections. Unimproved.
Patient 6 Male, white Age 43	Hands and wrists covered with red, weeping, crusted eczema. Foci on feet unnoticed.	12 years. No remissions for seven years.	Injections of trichophytin and oidiomycin.	Cured after 7 injections. No recurrence until 7 months later when patient developed spot on side of finger.

TABLE I—CONT'D

NAME	LOCALIZATION AND EXTENT	DURATION	PROCEDURE	RESULTS
Patient 7 Male, negro Age 49	Face, arms, hands, chest, ankles. Crusted, itching lesions.	15 years. Worse in summer. No remissions.	Injections of trichophytin and oidiomycin.	Practically cured after two treatments. Better than he had been in 15 years.
Patient 8 Female, white Age 37	Itching and breaking out of hands, palms and fingers. Worse in winter.	20 years. No remissions for several years past.	Injections of trichophytin and oidiomycin.	Cured after 24 treatments. Desensitization completed after hand cured. No return for 9 months.
Patient 9 Male, white Age 30	Patchy and rough, itching areas over body. Mild skin lesions between fingers.	6 weeks. 10 weeks.	Injections of trichophytin-Metz. Trichophytin-Lederle. Oidiomycin-Lederle.	Cured after three treatments.
Patient 10 Female, white Age 24	Eczema backs of both hands and fingers. Winters only.	21 years.	Injections of trichophytin and oidiomycin.	Unimproved.
Patient 11 Female, white Age 21	Small blistery lesions sides of fingers and hands.	7 years.	Injections of trichophytin and oidiomycin.	Cured after one treatment. No return in 12 months.
Patient 12 Female, white Age 29	Slight breaking out of hand.	6 months without remissions.	Injections of trichophytin and oidiomycin.	Cured after 4 treatments. Desensitization incomplete.

TABLE II

GROUP II

NAME	LOCALIZATION AND EXTENT	DURATION	PROCEDURE	RESULTS
Patient 13 Male, white Age 28	Eczema patches on arms and legs for two winters. Slight ringworm of feet several years, itching.	2 years. Winters only. Feet several years.	1. Removal important allergens. 2. Injections trichophytin and oidiomycin.	Injections completely cleared the skin. Desensitization incomplete. No recurrence 6 months later.
Patient 14 Female, white Age 39	Eczema of groins, inner surfaces of thighs, red, itchy and angry. Sides of fingers slight.	3 weeks on thighs, hands a longer time. Previous attacks groin and left breast 1 and 4 years ago.	1. Removed positive food as much as possible. 2. Injections of trichophytin and oidiomycin.	Rash dried up 2nd day after first injection. 20 injections gave complete relief. Desensitization incomplete. No recurrence 9 months later.
Patient 15 Male, white Age 22	Eczema, patchy, red and itchy areas both forearms and hands.	----------	1. Removed positive foods from diet. 2. Injections of trichophytin and oidiomycin. 3. Treated with dust.	Rash practically cleared after 26 treatments. Desensitization incomplete. Break of diet caused slight exacerbation repeatedly.
Patient 16 Female, white Age 45	Eczema, raw, red, palms of hands, between fingers, right side of neck, between toes.	4 years, constant.	1. Removal of positive allergens. 2. Injections of trichophytin and oidiomycin.	Severe general reactions. Improved 18 treatments. Skin almost cleared.
Patient 17 Female, white Age 22	Breaking out between fingers and on backs of hands and feet.	7 years, few short remissions.	1. Removal of positive foods from diet. 2. Injections of trichophytin and oidiomycin.	Cured after 26 treatments. No recurrence after 8 months. Desensitization complete.
Patient 18 Female, white Age 15	Dry, red, unsightly eruption backs of both hands, fingers and wrists.	2 years.	1. Removal of several foods. 2. Injections of trichophytin and oidiomycin.	Cured after 29 injections. Final dose: Trichophytin 1:30, 0.02 c.c. Oidiomycin 1:100, 0.02 c.c.
Patient 19 Female, white Age 37	Eczema, hands, fingers, neck and nails. Itchy, crusted.	5 years.	1. Elimination of positive foods and inhalants. 2. Injections of trichophytin and oidiomycin.	Almost cured except for a few raw places on fingers, as long as patient does not flagrantly break diet.

TABLE II—CONT'D

NAME	LOCALIZATION AND EXTENT	DURATION	PROCEDURE	RESULTS
Patient 20 Female, white Age 21	Eczema hands and feet, arms, legs, breasts and trunk. Very mild, itchy and patchy.	9 months.	1. Removal of positive allergens. 2. Injections of trichophytin and oidiomycin.	Cured after 19 treatments. Desensitization incomplete. No recurrence 7 months later.
Patient 21 Male, white Age 19	Eczema over whole body and face. Acute, red, itchy. Sometimes weeping, sometimes thick and dry.	4 years. Summer and fall, only. Constant past year. Improved in winter.	1. Removal of positive foods and inhalants. 2. Desensitization to grass and ragweed. 3. Injections of trichophytin and oidiomycin.	No improvement. Exacerbation after treatment of trichophytin 1:300, oidiomycin 1:1000. Patient became afraid of treatments and refused them.
Patient 22 Male, white Age 29	Eczema. Itchy and flaky. Eczema in front of ear and on neck. Ringworm between toes.	7 years. Worse in winter.	1. Removal of two positive foods from diet. 2. Injections of trichophytin and oidiomycin.	Cured.
Patient 23 Male, white Age 27	Itchy eczema of feet, thigh and inguinal regions.	----------	1. Injections of trichophytin and oidiomycin.	Cured. No recurrence several months later.
Patient 24 Female, white Age 32	Eczema on feet, over sides and top and left forearm. Rough and itchy.	----------	1. Removal of positive foods and inhalants. 2. Injections of trichophytin and oidiomycin.	Whitfield's ointment and x-ray therapy had never improved skin. Cleared after 16 injections.
Patient 25 Female, white Age 35	Spot of eczema on back of neck at hair line, about 3 cm. by 1 cm. Rough and itchy. Some eczema forearm, bottoms of feet.	Spot on neck several years. Generalized eczema few weeks.	1. Removal of positive foods and inhalants. 2. Injections of trichophytin and oidiomycin.	Unimproved. Desensitization incomplete.
Patient 26 Female, white Age 42	Hands, back of hands and fingers, halfway up forearm. Solid, crusted sore lesions.	12 years. Cleared in summer. Continuous for past year.	1. Removed wool, avoided pork. 2. Injections of trichophytin and oidiomycin.	Only slightly improved. Desensitization incomplete.

TABLE II—CONT'D

NAME	LOCALIZATION AND EXTENT	DURATION	PROCEDURE	RESULTS
Patient 27 Male, white Age 20	Itching, scattered lesions, feet and inguinal regions. Acne form lesions of face.	----------	1. Partial removal of positive foods. 2. Injections of trichophytin and oidiomycin.	Improvement doubtful. Patient still under treatment. Desensitization incomplete.
Patient 28 Female, white Age	Itching and breaking out of hands, especially palms. (Daughter of Patient 8.)	Few weeks.	1. Removal of positive allergens. 2. Injections of trichophytin and oidiomycin.	Cured.
Patient 29 Male, white Age 21	Small dry patch eczema, dorsum right thumb, occasional itching.	6 months.	1. Injections of trichophytin and oidiomycin.	Cured for few weeks. Returned. Treatment continued.
Patient 30 Female, white Age 54	Eczema; bright, red, patchy, itching volar surface, right forearm. Ulcers inside of mouth.	1 year.	1. Removal of positive allergens. 2. Injections of trichophytin and oidiomycin.	Cured after 6 treatments. Desensitization continued. No return after 4 months.
Patient 31 Female, white Age 19	Itching, eczema of neck. Mild and patchy. Ringworm of feet preceded onset one month.	4 months.	1. Removal of positive food and inhalants. 2. Injections of trichophytin and oidiomycin.	Improved. Stopped after 8 treatments. Desensitization incomplete.
Patient 32 Female, white Age 46	Unsightly, red, itching lesions 2½ inches in diameter on side of neck. Spot right on side of chest and left forearm. Slight ringworm between toes.	4 months without remission. Patient had had previous occurrences.	1. Removal of positive foods from diet. 2. Injections of trichophytin and oidiomycin.	Cured after 21 treatments. No return 9 months later. Desensitization incomplete.
Patient 33 Female, white Age 50	Slight itching, rough, eczema of hands and feet. Red, angry, itching, patchy eczema over arms, legs, thighs, breasts, and over most of ventral surfaces of body.	25 years. 6 weeks.	1. Removal of positive foods and inhalants. 2. Injections of trichophytin and oidiomycin.	Cured after many treatments. No return in 9 months. Final dose: Trichophytin 1:30 0.03 c.c. Oidiomycin 1:100 0.03 c.c.

"results" are indicated the presence and degree of improvement and the number of injections required to obtain this result. Also we have indicated "desensitization incomplete" where no attempt was made to bring the patient to a point where he was skin negative to the empirical dose of 0.1 c.c. of the usual testing dilutions of the extracts. Under "comments" is included any point of interest not contained in the preceding columns and some description of the course of the disease during this period of treatment. The occurrence of exacerbation of the lesions and increased sensitivity of the patient to the extract are described where these occurred during the course of treatment. Other known allergies in the patient are also indicated.

CONCLUSIONS

Many dermatoses, some of them resistant and of long standing, are due to hypersensitivity or allergy to fungi or their products. These are especially common in people who have other manifestations of allergy.

The important fungi involved belong to two groups: the monilia and the trichophytin groups. These are immunologically distinct; therefore both of them should be used in testing.

Positive diagnosis should be based upon the positive skin test plus an alteration of the clinical condition coincident with the height of the local reaction. The positive skin test is evidence only of previous infection.

The final therapeutic result is often influenced by the removal of other offending allergens.

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IS THERE AN INCREASED SUSPENSION STABILITY OF THE ERYTHROCYTES IN ALLERGIC DISEASE?*

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Schulhof[1] in 1933 reported that among the sedimentation rates of 610 routine unselected patients 115, or 19 per cent, had rates much slower than the normal rate expected in healthy subjects. Investigating these patients he found a high incidence of allergic manifestations among those with slow rates. He concludes that "a very slow sedimentation rate of the erythrocytes is a constant symptom in allergic individuals unless they show evidence of a complication, especially infection, which tends to accelerate the sedimentation."

If the findings of Schulhof are corroborated, the sedimentation rate should be a valuable aid in reaching a working diagnosis in some of the more obscure manifestations of allergy. It would, for example, help to distinguish the patients suffering from perennial allergic rhinitis simulating sinus infection from those with chronic infectious sinusitis. In this connection the sedimentation rate should be distinctly of value, since the x-ray examination frequently fails to differentiate the two conditions, the sinuses often appearing cloudy in the allergic as well as in the infectious type.

PRELIMINARY CONSIDERATIONS

A variability of the sedimentation rate of erythrocytes is not a new observation. The phenomenon of an increased rate of settling of the blood in inflammatory disease has been known to physicians for centuries and was considered a significant clinical sign. Galen was apparently the first to call attention to it under the name "Crusta phlogistica." John Hunter, in 1791, wrote concerning it, and many of his contemporaries regarded it as an important clinical fact. As their practice of blood letting was abandoned, the phenomenon was almost completely forgotten until its revival by Fahreus[2] in 1918. Numerous published articles in this country and abroad attest the interest of physiologist and clinician alike in this subject. Attempts to understand the principles underlying blood sedimentation and to explain variations in rate have brought forth various hypotheses, none of which has been generally accepted. From the clinical point of view an abnormal rate is generally interpreted as evidence of a disturbed

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physiologic condition of the blood occurring only in disease, a notable exception to this being pregnancy and the first few weeks of the puerperium. A rapid sedimentation rate is generally accepted as indicating a destructive process in the body. Cutler[3] makes the following classification of diseases in which the rate of sedimentation is increased: (1) chronic infectious diseases, such as tuberculosis and syphilis; (2) malignancy; (3) localized suppurations such as pelvic inflammations, suppurative mastoiditis, suppurative sinusitis, empyema of the gallbladder, bronchiectasis; (4) acute infectious diseases, such as pneumonia, septicemia, acute endocarditis, and the exanthems.

Among the conditions in which the sedimentation rates are unaltered Cutler lists the allergic diseases, asthma and hay fever. In this work, however, he had directed his attention to the discovery of conditions in which the rate of sedimentation was increased over normal. Caution should be exercised in accepting the results obtained by Storm Van Leeuwen,[4] who reported that 77 out of 85 patients had normal rates. What he really found was that eight of the 85 had increased rates of sedimentation and the remaining 77 had rates falling within limits generally accepted as normal. Until quite recently the possibility of a deviation from the normal on the slow side seems to have received no attention. In general, it may be said that patients have been classified either as normal or as fast. Sedimentation rates not increased were of no interest to earlier investigators, all such being relegated to the so-called normal group. Schulhof has called attention to investigations[5] dealing with clinical conditions which are the exceptions to this rule. These deal with sedimentation rates in serum disease. He also refers to experimental evidence[6] which points to an increased suspension stability in anaphylactic and peptone shock.

Schulhof criticizes the usual method of calculating sedimentation rates as follows: "Instead of taking the time as a measure of the speed of the sedimentation, the majority of the methods determine the distance reached by the column of plasma in a definite period, usually an hour. With Westergren's widely used technic a normal blood will show in one hour out of a column of blood 200 mm. long, only 3 mm. of clear plasma. While this method is excellently suited for the study of rapid sedimentations permitting a very wide range (about 100 mm.) for them, it is obvious that it fails with slow speeds which could be estimated only with great uncertainty with a range of 3 mm." The Linzenmaier method he thinks is better adapted to the discovery of slow rates, inasmuch as this measures the time required for the corpuscles to settle through a certain distance, arbitrarily established as 18 mm. He states that in health the time required for settling to this point is about 90 minutes in men and somewhat faster (he does not say how much) in women. In his study he chose arbitrarily 200

minutes as the lowest limit of a very slow rate. He states that some of the rates had to be extrapolated as it would have taken too long to watch the extremely slow sedimentation.

In our investigation, we did not consider it feasible to use the Linzenmaier technic, for to observe the exact time at which the column of settling corpuscles reached the mark it would require the constant attention of a technician. It seemed, however, that comparable data could be obtained by the Westergren technic if it were carried out with observations at intervals over a period of four hours instead of the standard technic of one-hour observation. This would carry the test to 240 minutes or 40 minutes beyond the arbitrary lowest limit set by Schulhof as a very slow rate. The only serious question that can be raised against the conclusions of Schulhof concerns the acceptance of his criteria of normals as sufficiently well established.

Since the beginning of this study additional reports have been published. Uffe[7] published the results of a study of 150 cases of allergic disease divided as follows: asthma, 108 cases; hay fever, 22 cases; asthma and hay fever combined, 14; urticaria, chronic allergic rhinitis, 4; allergic conjunctivitis, 1. He used the Cutler technic and divided his cases into four groups:

1. Slow, in which the rate was not more than 2 mm. by the first hour.
2. Normal, 3 to 10 mm.
3. Moderately fast, 11 mm. to 20 mm.
4. Fast, more than 20 mm. by the end of the first hour.

He reports 9.3 per cent of his series with slow rates; 48.7 per cent with normal rates; 32 per cent with moderately fast rates; and 10 per cent with definitely fast rates. The group consisted of 88 males and 62 females. None of the females had a slow rate. In drawing this conclusion, however, he made no distinction as to sex, a very serious error, since it has been repeatedly shown that the sedimentation is distinctly more rapid in the blood of normal females than is observed in the blood of normal males. Uffe made no attempt to compare his standard with that of Schulhof.

Westcott and Spain[8] reported results of a study done with the Westergren technic. Seventy-five cases in all were reported, comprising hay fever, asthma, and nonseasonal coryza. They concluded that "the erythrocyte sedimentation rate in uncomplicated noninfective asthma, hay fever or nonseasonal allergic coryza falls within the accepted normal limits and particularly in hay fever toward the low normal limit."

Very few data are given in their report. The same question must be raised concerning this report as regards that of Uffe in that no distinction was made between the sexes. Furthermore, it is a question in which the standard values used are correct. Their interpretation

of the normal rate included a range which may be too great, the term "normal" including cases falling between 2 mm. and 12 mm. per hour. Referring to Westergren's original publication, we find the following grouping for one-hour tests:

1. Under 1 mm: Subnormal rate (unqualifiedly so) in women; subnormal for men when the two-hour rate is less than 2 mm.
2. 1-3 mm: A safe normal for men; subnormal for women.
3. 4-7 mm: Upper limit of normal for men; safe normal for women.
4. 8-11 mm: Slightly pathologic for men; upper normal levels for women.

Fast rates: 8-11 mm. for men; 12-15 mm. for women.

Moderately fast: 30-35 mm.

Fast: 35+ mm.

Very fast: 80 mm.

Although Westergren mentions the possibility of a subnormal sedimentation rate, his studies do not include an investigation of this phenomenon. Obviously his chief interest and investigations were in rates faster than normal.

Reports in the literature indicate a fairly good agreement as to the differentiation between normal and fast rates, but references to rates slower than normal are conspicuously absent.

Cutler gave the normal sedimentation rate for men as from 2 mm. to 8 mm. in 60 minutes, with an average of from 3 mm. to 4 mm. For women his figures were from 2 mm. to 10 mm. with an average of from 5 mm. to 6 mm.

It is apparent from the foregoing that no generally accepted standard exists as to what constitutes a variation from normal on the slow side, since very few investigators have concerned themselves with that possibility.

Because of the absence of a standard we are obliged in this analysis to make comparisons with values which have been stated above.

HAY FEVER

Since hay fever is one of the most definite forms of allergic disease, it was chosen as a starting point for this study. Sedimentation rates were done by the Cutler technic on 30 male patients with this condition. Observations were made at fifteen-minute intervals for the first hour and then hourly through a period of four hours. The rates observed were even slower than those reported by Schulhof, for in no case was the mark 18 mm. reached in 240 minutes. The slowest rate was 2.5 mm. and the fastest 11 mm. for the four-hour period with the mean rate of 7.15 for the 30 cases (Table I).

The next 10 cases were run by the Cutler and Westergren methods simultaneously and observations made as before through a four-hour

TABLE I

SUMMARY OF SEDIMENTATION RATES IN HAY FEVER PATIENTS (MALES)

MINUTES	60		120		180		240	
	C	W	C	W	C	W	C	W
NUMBER OF PATIENTS	30	30	30	30	30	30	30	30
Mm. minimum	0.5	1.0	1.0	2.0	2.0	3.0	2.5	4.0
Mm. maximum	4.5	5.0	8.0	12.0	10.0	18.0	11.0	24.0
Mm. mean	1.76	2.75	4.15	6.53	5.66	11.33	7.53	15.48
P. E. of mean	±.13	±.13	±.24	±.36	±.29	±.50	±.32	±.70

C=Cutler technic.
W=Westergren technic.
P.E. = Probable error.

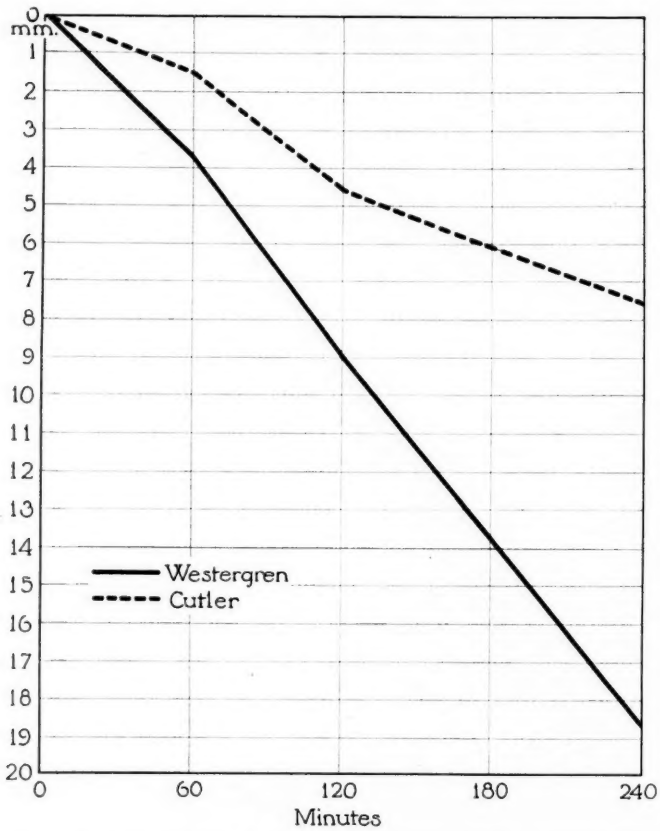

Fig. 1.—A comparison of the mean sedimentation rates of ten patients by the Cutler and Westergren methods run simultaneously on the same ten patients.

period. The rates of sedimentation in the two methods are obviously different (Fig. 1). Twenty additional cases, making 30 in all, were then done by the Westergren technic, using four-hour observations.

Since the tubes and dilutions are similar to those used by Schulhof and since our observations were over a four-hour period, it is possible to compare results. Using Schulhof's criteria for normals (18 mm. in 90 minutes) then all these patients had abnormally slow rates for

none reached the 18 mm. mark in 120 minutes, and only two had reached the 18 mm. mark in 180 minutes (Table I). Twenty-four patients (80 per cent) would be classified as having very slow rates, since the mark 18 mm. was either not reached or not exceeded in 240 minutes. These rates are slower than those reported by Schulhof, a fact which may be accounted for in that he made no sex distinction. Of the 20 female patients with hay fever on whom sedimentation rates were determined by the Westergren method (Table II), 10 patients would be classed as definitely slow according to Schulhof, since the mark 18 mm. was not reached in 120 minutes. Only four patients failed to reach the 18 mm. mark at the four-hour period.

TABLE II

SEDIMENTATION RATES IN HAY FEVER (FEMALES)

MINUTES	60		120		180		240	
	C	W	C	W	C	W	C	W
NUMBER OF PATIENTS	8	20	8	20	8	20	8	20
Mm. minimum	2.0	2.0	4.5	4.0	6.0	5.5	8.0	9.0
Mm. maximum	8.0	12.0	12.0	20.0	15.0	34.0	17.50	44.0
Mm. mean	5.43	7.22	9.5	15.35	11.93	22.75	13.62	27.65
P. E. of mean		±.54		.89		±1.31		±1.85

C=Cutler technic.
W=Westergren technic.
P.E. = Probable error.

Of the 30 male hay fever patients examined by the Cutler technic, 21 patients, or 70 per cent, are definitely slow in accordance with the criteria used by Uffe; i.e., having rates of less than 2 mm. during the first hour. If we class as slow all cases below 3 mm. for the first hour, this being the point which he used as normal, then 24 of our patients (80 per cent) have rates which would by Uffe have been adjudged slow. The remaining 6 patients would have to be adjudged slow normals, four cases having a rate of 3 mm. and two of 4 mm. Uffe did not discriminate between the sexes. In accordance with his criteria none of the females in our series had slow rates (Table II). Evaluating these rates done by the Cutler technic in terms of Cutler's own values, we find that 17 patients (56.6 per cent) have rates under 2 mm. and that 21 patients (70 per cent) have rates not exceeding 2 mm. for the first hour. The maximum of 4.5 mm. was attained by one patient and the mean rate for 10 patients is 1.76 mm. Recalling Cutler's averages of 3 mm. to 4 mm. for the one-hour period the mean of 1.76 appears significantly slow. For the females I have determined the rates on eight patients by the Cutler technic with a range from 2 mm. to 8 mm. and an average of 5.2 mm. corresponding very closely to Cutler's normal values for females.

Westcott and Spain state that all their patients had rates falling within accepted normal limits and the hay fever patients particularly

toward the low normal limit. According to Westergren's definition of a subnormal rate (less than 1 mm. for the first hour) all our patients have normal rates. Westcott and Spain, using the Westergren technic, defined normal limits from 2 mm. to 12 mm. per hour. In terms of these values none of our patients had slow rates, although all would group near the low normal side. These values, however, are distinctly at variance with those used by Uffe and as defined by Cutler, for a rate of 1.5 mm. for the first hour by the Cutler technic corresponds to approximately 3.75 mm. by the Westergren technic (Fig. 1); or, in other words, sedimentation by the Westergren technic is approximately two and one-half times as fast as it is by that of Cutler. If, then, a rate of 2 mm. or less by the Cutler is slow, it follows that rates under 5 mm. by the Westergren method are also slow.

Furthermore, it can be shown that this value corresponds to that used by Schulhof. In going over our patients we have found that those whose erythrocytes settled 18 mm. during 180 minutes have rates between 4 mm. and 5 mm. for the first hour. Recalling that the sedimentation time of 120 minutes for the fall of 18 mm. was considered slow, and that a sedimentation time of 200 mm. for the same distance was considered very slow by Schulhof, and if he be correct in his assumption that the normal value is 18 mm. in 90 minutes, then it follows necessarily that a rate below 5 mm. during the first hour should be considered slow and those below 4 mm. as very slow, since the former corresponds to a sedimentation time of 180 minutes and the latter to a sedimentation time of 240 minutes by the Linzenmaier method as reported by Schulhof. If we use these corrected values for the Westergren method to bring it into conformity with values stated by Cutler, then all our male hay fever patients have slow sedimentation rates, for none exceeded 5 mm. in one hour and only one patient of 30 patients reached that level. The mean for the 30 male patients for the first hour was 2.75 mm. It is obvious that the criteria of Westcott and Spain are at variance with those used by Uffe. On the other hand, the values of Uffe and those of Schulhof correspond very closely.

Gelfand and Victor[9] reported on sedimentation rates by Cutler's method in 63 hay fever patients, for whom they observed normal rates with the exception of 11 patients who had fast rates. In all except one of the 11 patients an infectious process was demonstrated. In conclusion they state: "The sedimentation rate of the red blood cells in patients with hay fever, who present no other pathologic complications of a destructive nature, remains normal before as well as during the hay fever season. . . . ." They report only 2 out of 61 hay fever patients having rates adjudged slow. The criteria used by them were essentially the same as those used by Uffe, and our discussion of his report also applies here.

ASTHMA

Sedimentation rates were obtained for 20 males and 10 females with asthma, all of which were done by the Westergren method with four-hour observations. The results are shown in Table III. Uffe reported only 5 patients of 108 patients as having slow rates. Westcott and Spain reported 10 patients with asthma, without infection, as having rates between 3 mm. and 10 mm. for the first hour by the Westergren technic. Again they make no reference as to sex, a serious error. Our 20 male asthma patients have rates very similar to those of the hay fever patients (Table III).

TABLE III

SEDIMENTATION RATES IN ASTHMA (WESTERGREN METHOD)

	20 MALES				10 FEMALES			
MINUTES	60	120	180	240	60	120	180	240
Mm. minimum	1.0	2.0	2.0	2.5	0	1.5	2.5	3.0
Mm. maximum	5.0	11.0	18.0	24.0	12.0	22.0	24.0	46.0
Mm. mean	2.85	6.87	10.7	14.67	6.0	13.05	17.95	23.2
P. E. of mean	±.17	±.43	±.66	±.91	±.88	±1.51	±2.29	±3.21

P.E. = Probable error.

The highest rate in two hours was 11 mm., and no patient exceeded the mark 18 mm. in three hours. By Schulhof's definition these are all very slow. If the limits of a normal rate are 2 mm. to 12 mm. as used by Westcott and Spain, then the rates of these patients are segregated on the low side of the normal rate. The mean of the asthma series is 2.85 mm. for the one-hour period.

It is inconceivable that so large a group could by chance be grouped entirely toward the slow side of the normal without exception. Again the mean of these patients, 2.85 mm., corresponds to that of 1.25 mm. to 1 mm. by the Cutler technic (Fig. 1). Obviously the weight of evidence points to the fact of a sedimentation rate slower than normal in this group of asthmatic individuals.

Of the female asthma patients one had an unusual suspension stability remaining unchanged for the first hour and settled only 3 mm. during the four-hour period. Four other patients had rates of less than 18 mm. in four hours. Three of these settled only 2 mm. during the first hour, one other to the level of 5 mm. These five patients (50 per cent) of the females are definitely slow according to the criteria of Schulhof, and would also fall into the slow group of Uffe, since the rates correspond to 2 mm. or less by the Cutler method. The remaining five patients (50 per cent) had rates ranging from 7 mm. to 12 mm., the probable normal values. A greater variability of sedimentation rates is observed among the females.

PERENNIAL ALLERGIC RHINITIS

Sedimentation rates were obtained for 55 males and 20 females. Five of the females had positive evidence of infection, pus having been washed from the antrums by puncture and irrigation. These five were therefore excluded from the series when tabulating the results (Table IV).

TABLE IV

SEDIMENTATION RATES IN PERENNIAL ALLERGIC RHINITIS (WESTERGREN METHOD)

	55 MALES				15 FEMALES			
MINUTES	60	120	180	240	60	120	180	240
Mm. minimum	1.0	2.0	2.0	4.0	2.0	4.0	6.0	7.0
Mm. maximum	5.5	12.0	22.0	28.0	9.0	22.0	34.0	43.0
Mm. mean	2.72	6.03	9.97	13.4	6.9	15.4	22.93	30.0
P. E. of mean	±.11	±.26	±.44	±.57	±.50	±.98	±1.49	±1.95

P.E. = Probable error.

The sedimentation rates for the male patients with nonseasonal allergic coryza are very similar to those of hay fever and asthma. The range for 55 patients is from 1 mm. to 5.5 mm. with a mean of 2.72 mm. for the one-hour period. Twelve patients (21.8 per cent) had rates of less than 2 mm. for the first hour. These would correspond to rates of 0 to 1 mm. by the Cutler method and all unquestionably slower than expected in normals. Twenty-six (47 per cent) additional patients had rates of from 2 mm. to 3 mm., which would correspond to Cutler values of less than 2 mm., bringing the total of slow cases, as defined by Uffe, to 38 (68.8 per cent). The entire series would be slow according to Schulhof's criteria, since none attained the level of 18 mm. in the two-hour period. All but seven of the patients (87.2 per cent) would have been rated as *very* slow, since the 18 mm. mark was not reached in three hours. The rates of all patients of this series fall on the slow side if the normal distribution of 2 mm. to 12 mm. is accepted. It appears then that this entire series of male patients with allergic coryza have a greater suspension stability of the erythrocytes than that generally stated for normal individuals.

The sedimentation rates in the female patients with allergic coryza are much faster than those in the males; the rates are apparently not slow as judged by the standards generally accepted and discussed in the foregoing. For the first hour period the range is from 2 mm. to 9 mm. with a mean of 6.9 mm. It is to be noted, however, that none of the patients attain the upper normal limits. Schulhof, as we have stated, made no distinction between males and females. However, 10 (66.6 per cent) of these patients did not exceed the 18 mm. mark in two hours and would have been classified by him as slow.

SUMMARY

The data presented in this study seem to support the idea of an increased suspension stability of the erythrocytes in allergic disease.

The sedimentation rates of all patients with allergic disease used in this study are all slower than the theoretical mean of the range, 2 mm. to 12 mm. in one hour, generally accepted for normals, nor do they spread over this range.

It seems probable that the accepted normal range has been established from studies which did not exclude allergic individuals. The probability that this is true receives support by Vaughan's[10] recent report that upward of 10 per cent of the population develop major allergic manifestations and approximately an additional 50 per cent give a history of minor episodic allergic manifestations in the past.

Further investigation of normal rates is needed, with care to exclude those individuals with major and minor allergies in the personal history as well as those who have close relatives so affected.

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Special Reports

BUCKWHEAT SENSITIVITY*

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BUCKWHEAT[1] belongs to the *Polygonacea* or buckwheat family and is not a true cereal since it does not belong to the *Graminiae,* or grain family. Its products have long been considered excellent foods for both human and animal consumption. It is in general the best grain crop for poor thin land, and in the United States more than 60 per cent of the entire crop is produced in Pennsylvania and New York.

The outer hulls of buckwheat removed in milling are used as fuel, as packing material for bottled goods and bulbs, and to some extent for mixing into stock feeds.

The middlings consist of that portion of the grain just beneath the hull, and because of their high content of protein, carbohydrate, fat and mineral, they serve as a valuable food for cattle, producing a large flow of milk. They are useful as fertilizer because of the high nitrogen, phosphorus and potassium content.

The whole buckwheat grain is a good poultry feed. The straw is sometimes used for feed and is more readily eaten by stock if well preserved. It makes good bedding for cattle and also makes good manure.

The flour[2] is used extensively for making griddle cakes, and is found in the following prepared flours: Aunt Jemima Buckwheat, corn and wheat flour; H. O. Buckwheat Pancake Flour, corn meal, and wheat flour; Uncle Jerry's New England Self-Raising Flour, wheat and corn flour.

The flowers of buckwheat furnish an excellent source of honey for bees, but this product is usually a local one.

The earliest reference to a case of buckwheat sensitivity was a report by Smith[3] in 1909. He accurately described a case of angioneurotic edema and urticaria in a young man upon ingestion of small amounts of buckwheat, and violent asthma on the ingestion of moderate quantities of buckwheat. Symptoms of vasomotor rhinitis were manifested upon inhalation of extremely small quantities of buckwheat flour. Conjunctivitis and increased flow of saliva followed the application of small amounts of buckwheat to the tongue, and the

*From Allergic Clinics of Mount Sinai and Temple University Hospitals.

patient utilized this as a test for the detection of buckwheat in his food. Smith utilized the scratch, nasal, and oral tests for the presence of sensitivity in this individual. The skin reaction was so marked that it was followed by a constitutional reaction in the form of asthma. Highman and Michael[4] reported a case of urticaria due to buckwheat sensitivity with a positive scratch test and an amelioration of hives upon the elimination of buckwheat from the diet. Rowe[5] found 27 positive reactors in 500 consecutive cases of allergy, 22 patients giving a one-plus reaction, 4 yielding a two-plus and 1 a three-plus reaction. He estimated that 1 per cent of patients are clinically sensitive to buckwheat.

When white-colored animals[1] are fed large quantities of buckwheat over long periods of time and are then exposed to light, they develop a rash on their skin. The subject of this phenomenon, called photosensitization, or fagopyrismus, has been fully investigated by Sheard, Caylor and Schlotthauer.[6] Among the lower animals, the white or spotted guinea pigs, white swine and goats exhibit photosensitization, the degree of sensitivity being in the order given. The manifest symptoms closely resemble those of anaphylaxis, namely, agitation, itching, scratching of the ears, weakness, urticaria with sloughing, conjunctivitis, laryngitis, and bronchitis. Microscopic examination showed the lack of marked pathologic changes except those due to profound toxemia, i.e., petechial hemorrhages into all the viscera. Using the spectrophotometric method Sheard and his associates concluded that phyloporphyrin and cholehematin are the probable causes of photosensitization induced by buckwheat, and the hematoporphyrin and the fluorescence of chlorophyll are, in all probability, not the cause of it. Blum[7] in considering photosensitization stated that hematoporphyrin occurring in the body as uroporphyrin and stercoporphyrin has long been considered the sensitizing agent but concluded that it either plays no rôle in photosensitization or is the result of skin destruction. Taussig[8] corroborated the opinion of Blum by considering the porphyrins the result of sensitivity rather than the sensitizers. Blum contended that in man there are no examples of photosensitization resulting from ingested food. In this series of 19 cases of buckwheat sensitivity (8 patients clinically sensitive) it is interesting to note that not one case of photosensitization was encountered, this being in agreement with the work of Blum.

According to Vaughan's[9] botanical classification, buckwheat and rhubarb belong to the same family. Of the 19 cases of buckwheat sensitivity reviewed one patient gave positive skin reactions and none gave a history suggesting rhubarb sensitivity. It is therefore apparent that while these substances are botanically related, they bear no relation to each other so far as skin or clinical sensitivity is concerned.

In a review of 500 consecutive allergic patients who had extensive skin tests performed by the intradermal method, I was able to find only 19 who gave positive reactions to a buckwheat extract containing 0.01 mg. of total nitrogen per c.c. Of these 19 patients 8 were clinically sensitive to buckwheat; 6 had bronchial asthma, the result of inhalation coincident with their occupation as bakers. One had symptoms of allergic coryza upon inhalation; one manifested food allergy in the form of nausea, abdominal cramps, vomiting, and generalized pruritus upon ingestion of small quantities of buckwheat. Of the 6 having symptoms upon the inhalation of buckwheat, only one gave symptoms upon its ingestion.

Most patients with positive skin reactions to buckwheat also showed reactions to most of the cereals and to pollens, particularly to rye, timothy, and ragweed. Twelve of the 19 were clinically sensitive to ragweed (see Table I). This may be expected when one considers that the *Polygonaceae* are classified as weeds by Thommen,[2] despite their close relationship to the true cereals which usually show concomitant grass sensitivity.

Passive transfer to buckwheat by the Prausnitz-Küstner method was attempted on 12 patients, and 10 showed positive reactions. Of these, 8 were clinically sensitive to buckwheat.

In two male patients associated with the baking industry (Cases 17 and 19) where a change of occupation was not feasible, I attempted to hyposensitize them by the subcutaneous injection of buckwheat extract at weekly intervals beginning with a dose of 0.0001 mg. of nitrogen and gradually increasing until a marked local reaction occurred at the site of injection. This dose was maintained at weekly intervals until the clinical condition improved, at which time the interval between injections was lengthened.

Case 19 was that of a baker, aged thirty-eight years, who had had rhinitis upon exposure to buckwheat flour in 1920. A submucous resection at that time cleared up all symptoms. In July, 1933, he began having attacks of asthma, more marked while at or near his place of occupation. Buckwheat when eaten by mouth was innocuous. Hyposensitization was begun in September, 1933, and continued for a period of six months without any apparent benefit. Only one constitutional reaction was encountered, and this occurred when a change was made from a weaker to stronger extract, although the amount of nitrogen injected was the same in each. When the higher doses were reached, a marked local reaction was encountered, which was very painful and persisted for as long as three days. This was considered by the patient an annoying feature of the treatment.

In Case 17, the patient, aged 30 years, a driver-salesman on a bakery wagon for seven years, first noticed symptoms of rhinitis in July,

TABLE I

SKIN REACTIONS IN 19 BUCKWHEAT-SENSITIVE PATIENTS

CASE No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	TOTALS	CLINICALLY SENSITIVE
Buckwheat	+	++	±	+	+	+	++	+±	+	+	+±	+	++	+	++	+++	+++	++	+++	19	8
Corn	–	+	–	–	±	–	–	–	–	–	++	±	–	++	+++	+±	±	–	–	8	1
Rice	±	–	–	–	+±	–	–	±	–	–	–	+	+	–	++	++	+±	–	±	9	0
Rye	±	+	+	++	–	–	±	±	±	–	+	++	–	+	+±	++	++±	++±	++±	15	5
Oats	–	–	+	±	–	–	–	–	–	–	++±	++	–	±	++	+±	–	–	+	8	1
Barley	–	–	–	++	+±	–	+	–	–	+	+++	+±	–	++	++	+±	+++	++	+±	12	2
Wheat	+±	±	++	++±	–	±	–	±	–	–	+	–	–	–	++	+	–	–	+±	10	5
Ragweed	+++	++	+++	+++	+++	++	+++	+	+++	–	++	+	–	±	+++	+++	+++	+++	–	16	12
Timothy	–	+	+++	+	+	+	+	–	–	–	+	±	–	–	++	++	+	+	–	12	2
Rhubarb	+±	-	–	-	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	0

± = questionable reaction.
+ = slight reaction.
+± = slight plus reaction.
++ = moderate reaction.
++± = moderate plus reaction.
+++ = marked reaction.

1932. These occurred while he was on his route and particularly when he created a cloud of flour dust by moving baskets in the rear of the truck, or upon entering the shop where bread was made Hyposensitization was begun in November, 1933, and continued for five months. At this time treatment was discontinued because of complete relief. Marked local but no constitutional reactions as in the previous case were noted. The patient was allowed to remain at his occupation, with a warning that symptoms would probably recur, but it is almost one and one-half years since the last treatment and he remains symptom-free.

CONCLUSIONS

Five hundred consecutive cases of allergy which had detailed studies were reviewed, and 19 patients (4.8 per cent) were found skin sensitive to buckwheat. Only 8 were clinically sensitive, a percentage of 1.6. Most of the latter reacted after inhalation and showed no untoward signs or symptoms upon buckwheat ingestion. Most cases of buckwheat sensitivity were found in bakers or those associated in some way with the baking industry and in housewives who do considerable baking.

Photosensitization and fagopyrismus were briefly reviewed, and the conclusion reached that buckwheat plays no rôle in human photosensitization. The porphyrins that are frequently found in cases of photosensitization are considered the result of sensitization and not the sensitizers.

The coexistence of buckwheat and ragweed sensitivity in 12 out of 19 cases together with the fact that buckwheat is classified as a weed shows the close relationship between these two substances. Neutralization in vitro in three cases disclosed the fact that there was no antigenic relationship between ragweed and buckwheat.

Attempts at hyposensitization were carried out in two cases, one a case of baker's asthma, with little or no success, and the second a case of allergic coryza due to buckwheat, with good results. Desensitization with an atopen as powerful as buckwheat is still a dangerous weapon in the hands of untrained men and should always be used with caution, especially since the result in the patient with baker's asthma was not followed by sufficient improvement to warrant its use in other similar cases. In the patient with allergic rhinitis who was treated, the result was extremely gratifying, but the fear that this patient would suffer a relapse or even a progression to actual bronchial asthma prompted the advice of a change of occupation. Because of poor results in the treatment of occupational allergies associated with the baking industry and a tendency on the part of these individuals to develop severe and intractable asthma, it seems advisable to urge these patients to change their occupation.

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POLLEN SURVEY IN PITTSBURGH, PENNSYLVANIA, 1934*

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THE purpose of this report is to indicate the incidence of hay fever producing pollen for the Pittsburgh, Pennsylvania, district during the 1934 season. The survey does not include tree pollen. For the purpose of this paper, exposures are reported in three different locations, and the slides were counted and checked by three observers.

Microscope slides (3 in. × 1 in.), having been covered with a very thin film of white vaseline, were exposed to the air for twenty-four-hour periods in three different parts of the city. One slide was exposed in a district near one of the city parks, another in a residential district several miles away, and the third in one of the suburbs eight miles from the center of the city. The slides were placed in cardboard, gable-roofed receptacles, the front and back of the receptacles being open to the north and south respectively. The roof of each receptacle was about three inches above the slide. In this manner, free circulation of air is permitted, and the slides are protected from rain and excess dust.

The slides were usually examined within forty-eight hours after exposure. Examination was made with a microscope having a tube length of 160 mm., a 10X ocular, and a 10X objective. The number of ragweed pollen grains found in the examination of five widths of a slide corresponded to the number of pollen grains in one cubic yard of air. The count for other pollen is expressed in terms of grains per unit slide area. Camera lucida drawings were made of each new pollen found, and an attempt was made to identify them.

Since we are concerned with only those plants which are specific for hay fever, the survey is narrowed down to the three families whose pollen grains are certain of identification. Counts were made, however, of other pollens, which, as yet, have not been identified. The present survey includes three families.

I. *Gramineae* (grasses). The pollen grains of the different grasses are very similar in appearance under the microscope, and for this reason a separate count is not made for each grass; therefore they are all placed together under one heading.

This group includes among other grasses the following specific plants:

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(1) Canada blue grass (*Poa compressa*),—July; (2) June grass (*Poa pratensis*), May—July; (3) orchard grass (*Dactylis glomerata*), May—July; (4) red top (*Agrostis alba*), June—July; (5) timothy (*Phleum pratense*), June—July.

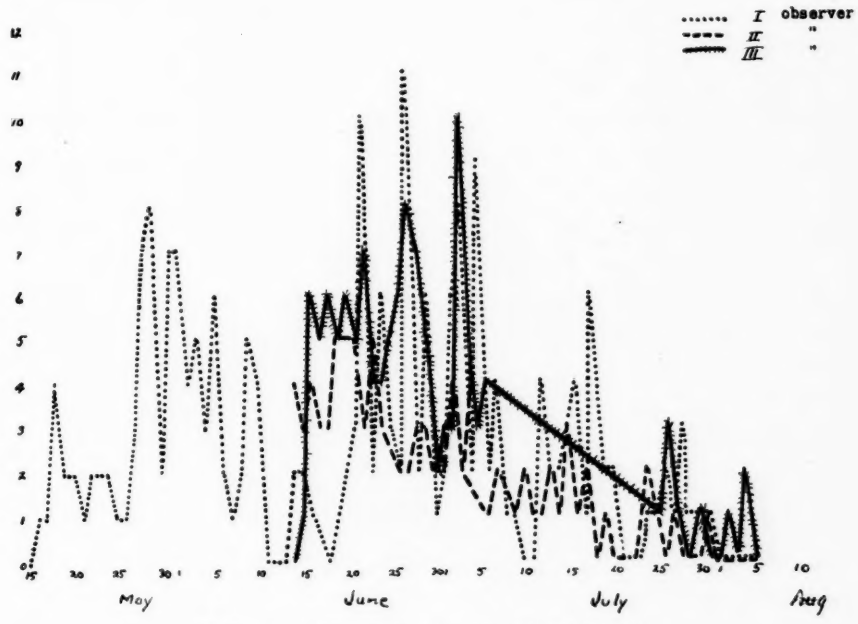

Chart 1.—Occurrence of grass pollen in 1934.

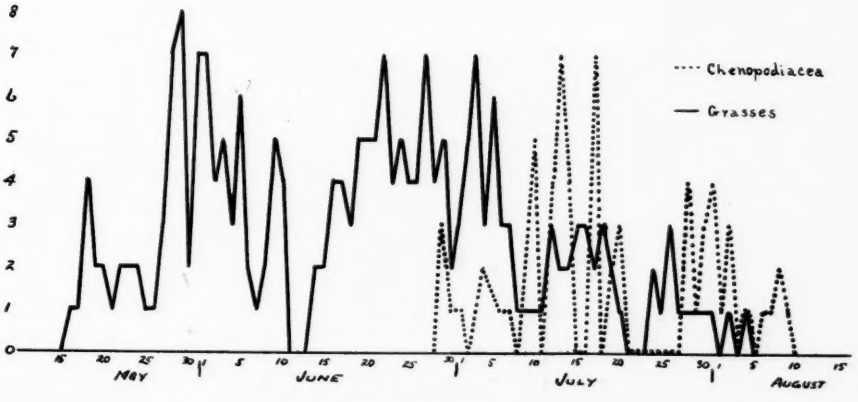

Chart 2.—Average pollen occurrence in 1934.

The average of the three counts shows that the greatest pollination of the grasses takes place between June 15 and July 7. During this time, however, there are fluctuations in the count due to meteorologic conditions, such as wind velocity, temperature, humidity, etc. Gen-

erally speaking, the count was low, and this corresponds with the clinical picture presented by untreated hay fever patients. The 1934 grass season was rather light. (Charts 1 and 2. Table I.)

TABLE I

GRASSES (POLLEN GRAINS PER UNIT SLIDE AREA)

1934	I	II	III	AV.	1934	I	II	III	AV.
May 13	0				July 1	2	2	3	3
14	0				2	6	4	10	5
15	0				3	8	2	4	7
16	1				4	2	–	3	3
17	1				5	9	–	4	6
18	4				6	2	1	–	3
19	2				7	4	2	–	3
20	2				8	1	–	–	1
21	1				9	1	1	–	1
22	2				10	0	2	–	1
23	2				11	0	1	–	1
24	2				12	4	1	–	3
25	1				13	2	2	–	2
26	1				14	2	1	–	2
27	3				15	3	3	–	3
28	7				16	4	1	–	3
29	8				17	1	2	–	2
30	2				18	6	0	–	3
31	7				19	2	1	–	2
June 1	7				20	2	0	–	1
2	4				21	0	0	–	0
3	5				22	0	0	–	0
4	3				23	0	0	–	0
5	6				24	1	2	–	2
6	2				25	1	1	1	1
7	1				26	2	0	3	3
8	2				27	1	1	1	1
9	5				28	3	0	0	1
10	4				29	1	0	1	1
11	0				30	1	0	1	1
12	0				31	1	1	0	1
13	0				Aug. 1	0	0	0	0
14	2	4	0	2	2	0	0	1	1
15	2	3	1	2	3	0	0	0	0
16	1	4	6	4	4	0	0	2	1
17	–	3	5	4	5	0	0	0	0
18	0	3	6	3					
19	–	5	5	5					
20	2	5	6	5					
21	3	5	5	5					
22	10	3	7	7					
23	2	5	4	4					
24	6	3	4	5					
25	3	–	5	4					
26	2	2	6	4					
27	11	2	8	7					
28	2	3	7	4					
29	6	3	5	5					
30	1	2	2	2					

II. *Chenopodiaceae.* This group includes chiefly Lamb's-quarters, or goosefoot (*Chenopodium album*), June to October (Chart 2, Table II.)

III. *Ambrosiaceae.* This group includes: (1) giant ragweed (*Ambrosia trifida*), August—October; and (2) low ragweed (*Ambrosia elatior*), August—October.

TABLE II

CHENOPODIACEAE (LAMB'S-QUARTERS)

1934	I	II	AV.
June 29	5	0	3
30	2	0	1
July 1	1	0	1
2	0	0	0
3	2	0	1
4	–	2	2
5	–	–	–
6	1	–	1
7	1	–	1
8	0	–	0
9	–	–	–
10	5	–	5
11	0	–	0
12	4	–	4
13	7	–	7
14	4	–	4
15	0	–	0
16	0	–	0
17	7	–	7
18	0	–	0
19	2	–	2
20	3	–	3
21	0	–	0
22	0	–	0
23	0	–	0
24	0	–	0
25	0	–	0
26	0	–	0
27	0	–	0
28	4	–	4
29	1	–	1
30	3	–	3
31	4	–	4
Aug. 1	1	–	1
2	3	–	3
3	0	–	0
4	1	–	1
5	0	–	0
6	1	–	1
7	1	–	1
8	2	–	2
9	1	–	1
10	1	–	1
11	1	–	1
12	0	–	0

I=counts made by one observer.
II=counts made by the other observer.
Av.=average of the two counts.
–=a count not made.
0=count made, but no pollen found.

The first ragweed pollen was observed on July 30. From this date to September 1, there was almost a steady increase in number (from 1 on July 30 to 291 on September 1). From September 2 to the mid-

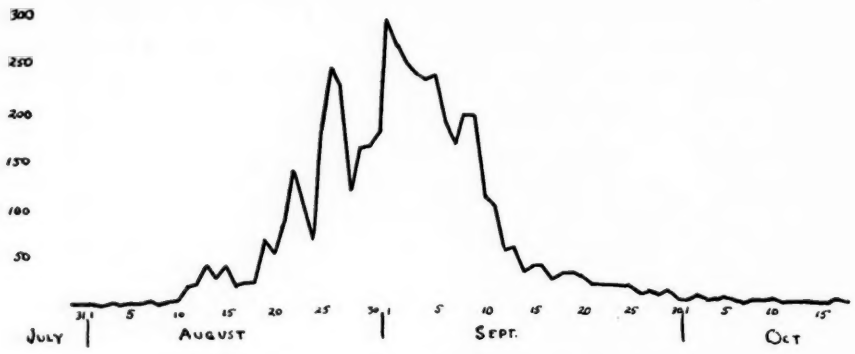

Chart 3.—Average ragweed pollen occurrence in 1934.

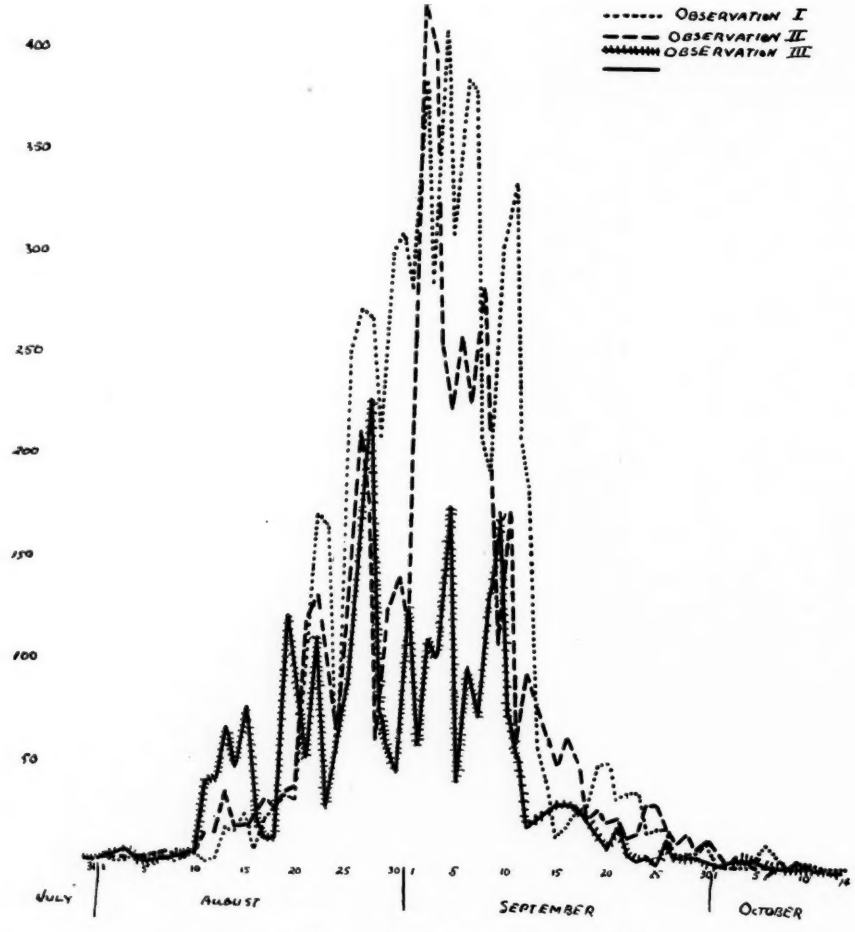

Chart 4.—Ragweed pollen occurrence in 1934.

dle of October the number declined, with fluctuations in number occurring throughout. (Charts 3 and 4. Table III.)

TABLE III

GIANT AND LOW RAGWEED

1934	I	II	III	AV.	1934	I	II	III	AV.
July 30	–	0	1	1	Sept. 9	305	109	170	195
31	–	0	1	1	10	334	172	74	194
Aug. 1	1	4	–	3	11	210	60	53	108
2	0	2	–	1	12	184	94	18	99
3	0	1	7	4	13	62	80	22	53
4	1	1	–	1	14	43	65	60	56
5	0	2	2	2	15	13	50	30	31
6	1	2	–	2	16	18	63	30	37
7	2	5	1	3	17	26	52	29	36
8	0	4	–	2	18	26	22	–	23
9	2	3	2	3	19	48	28	–	26
10	4	5	–	5	20	52	21	9	28
11	0	14	40	18	21	34	23	18	25
12	7	15	42	22	22	36	13	6	19
13	18	35	66	40	23	37	15	2	18
14	15	19	49	28	24	16	30	5	17
15	24	19	77	40	25	17	28	1	16
16	13	23	23	20	26	19	17	12	16
17	22	31	13	22	27	7	12	6	9
18	28	28	13	23	28	7	16	7	10
19	34	33	122	63	29	8	9	5	8
20	31	35	91	53	30	11	11	–	11
21	–	117	52	85	Oct. 1	2	8	1	4
22	173	133	111	139	2	0	3	2	2
23	165	–	29	97	3	–	5	5	5
24	71	69	61	67	4	0	2	0	2
25	250	207	88	182	5	5	0	3	4
26	273	214	–	244	6	11	1	0	6
27	270	177	229	226	7	5	0	0	2
28	210	62	80	118	8	0	0	0	0
29	300	126	58	162	9	1	0	2	2
30	310	140	46	164	10	2	0	0	1
31	284	117	125	176	11	1	0	1	1
Sept. 1	390	422	60	291	12	0	0	0	0
2	286	400	110	266	13	0	0	0	0
3	410	231	104	249	14	0	0	0	0
4	310	224	175	237	15	0	0	0	0
5	387	259	42	230	16	0	0	0	0
6	380	226	96	234	17	0	0	0	0
7	210	283	73	189	18	0	0	2	1
8	194	–	134	164	19	0	0	0	0

A personal element enters into the counts. Checks were made on one another's counts by these observers, and the differences of the two counts range from a very small percentage of difference to about 300 per cent. This difference is found to depend on the locus of exposure. It is found that where one observer would find clumps of pollen grains and as a result obtain a high count, the other observer, on counting the same slide, might examine portions of the slide in which these clumps did not occur, and so obtain a low count. Hence, the particular field examined is an important factor.

The authors are deeply grateful to Mr. O. C. Durham for many helpful suggestions and criticisms in the preparation of this paper.

Society Proceedings

COMBINED MEETING OF THE ASSOCIATION FOR THE STUDY OF ALLERGY AND THE SOCIETY FOR THE STUDY OF ASTHMA AND ALLIED CONDITIONS

ATLANTIC CITY, N. J., JUNE 10 AND 11, 1935

Treatment of Pollen Hay Fever and Rhinitis by Ionization of the Mucous Membrane. HUGH O. HOUSE, AND LESLIE N. GAY, Baltimore, Md. (Abstract.)

Twenty patients were treated by nasal ionization by the method of Warwick, whose instrument and exact technic were used. These patients included eight with hay fever (ragweed) and pollen asthma; three with allergic rhinitis; four with perennial nonallergic rhinitis; two with asthmatic bronchitis of bacterial (intrinsic) etiology; and three with allergic bronchial asthma. Each was thoroughly studied before treatment, and each was a typical example of the group represented.

Results are recorded in Table I.

TABLE I

RESULTS FROM IONIZATION

DIAGNOSIS	NO. CASES	IMPROVED	UNIMPROVED	WORSE
Hay fever	4	1	1	2
Hay fever and pollen asthma	4	1	2	1
Allergic rhinitis	4	1	2	1
Nonallergic rhinitis	3	2	1	
Asthmatic bronchitis	2	1		1
Bronchial asthma	3		2	1
Totals	20	6 (30%)	8 (40%)	6 (30%)
Nonallergic	5	3 (60%)	1 (20%)	1 (20%)
Allergic	15	3 (20%)	7 (47%)	5 (33%)

Of the five nonallergic patients three were improved, one was unimproved, and one was worse. Of the 15 allergic patients, three were improved, seven were unimproved, and five were worse.

Patients reacted to the treatment with varying degrees of severity. Three had but a slight reaction manifested by nasal obstruction without headache or other discomfort. Ten experienced a moderate reaction characterized by nasal discomfort, headache, and severe malaise lasting three to seven days. Seven suffered a severe reaction with nasal distress for a week or longer, accompanied by asthmatic manifestations.

It is concluded that the method has been helpful in several of the group of patients with nonallergic rhinitis. It was of no aid in the allergic group. In fact, many of the patients were made worse in that asthmatic manifestations followed treatment. The procedure often caused great local discomfort. In conditions of hypersensitiveness it should be given no consideration whatever.

Nasal Ionization—Preliminary Observations. H. L. ALEXANDER, St. Louis. (Abstract.)

The subject is being investigated in the Department of Otolaryngology of Washington University Medical School, where it has been separated into two phases: (1) a study of the mechanism by which relief occurs, and (2) a clinical analysis of the cases treated.

In patients in whom therapeutic results were obtained, the appearance of the lesion—a coagulation necrosis of the superficial cells—suggested that success was due to the same factors that operate when the nose is cauterized by carbolic acid, trichloracetic acid, diathermy, or actual cautery, for all of which successful results have been claimed. Such an assumption, however, becomes unlikely because of the observation that after ionizing one side of the nose, definite and sometimes striking improvement occurs on the other un-ionized side, simultaneously. Moreover, with the clearing of one side, a thickened sinus membrane opaque to x-ray examination showed marked clearing. A suspicion was thereby aroused that zinc ions may penetrate into the blood stream and have a remote effect. In this regard it may be of some significance that within a minute after the current is turned on patients may complain of a metallic taste. This short interval corresponds to that obtained between the intravenous introduction of bitter or sweet drugs and the instant they are tasted—a measure of the circulation rate.

It has been suggested that beneficial effects may be the result of the anesthetic rather than the metallic solution, as iontophoresis with cocaine and with adrenalin has had marked therapeutic effects in other allergic conditions. This is unlikely since children ionized under ether anesthesia have had the same therapeutic results as adults under cocaine. Whether or not zinc ions have a direct effect on the capillaries by interfering with their normal vasomotor action, as is the case with iontophoresis with histamine, beta-methyl-acetylcholyn, and other drugs, is as yet undetermined. This and other possible mechanisms are being investigated.

Histologic examination of the ionized mucous membrane shows signs of acute inflammation immediately after the treatment. The only permanent change is a little fibrosis which, however, is not enough to interfere with function. Each ionization, however, increases the

amount of fibrosis so that if done too often there is risk of significant damage.

From an immunologic standpoint, it was interesting that soon after restoration of the membrane to normal, topical application of the allergen caused little or no reaction, whereas previous to ionization it had a violent effect. In patients with reagins in the blood, the mucous membranes became resensitized in a short time and reacted as before to topical applications. Those without reagins remained insensitive as a rule for much longer periods of time. Iontophoresis was not observed to have any effect on the skin reactions to which the nasal mucosa was sensitive nor to reagins of such allergens.

From the clinical standpoint the following impression was gained: In hay fever, patients ionized before the season do poorly. If so treated during the season, success is measured largely by the duration of the season after the date of treatment. If performed late enough that when its effect wears off the season is over, it has all the appearance of a cure. In the great majority of cases, hyposensitization with pollen extract appears a superior method of treatment. In three patients with hay fever who were ionized, nasal symptoms disappeared but asthma occurred for the first time.

In vasomotor rhinitis, too, when a marked hypersensitiveness to a given allergen can be demonstrated, and when reagins are present, relapses occur. On the other hand, in the group of patients in whom no specific allergen could be demonstrated, nasal ionization has proved to be very effective. Some patients with this condition, whose symptoms were continual, have been completely relieved. A few of these have been well for one year. It is in this group that ionization seems to have its place, and it is considered a valuable and welcome form of therapy.

Success with asthma, vasomotor rhinitis, migraine, and vernal catarrh has been claimed. Many such patients have been seen, and the impression has been gained that ionization did no good.

From these observations it appears that the mechanism of relief from ionization as well as the factors entering the problem are as yet quite unknown. From a clinical standpoint it seems an inferior form of treatment for hay fever, asthma, and other allergic conditions with the exception of certain cases of vasomotor rhinitis without established etiology. Here it seems to have a definite place.

DISCUSSION ON "THE TREATMENT OF POLLEN HAY FEVER AND RHINITIS BY IONIZATION OF THE MUCOUS MEMBRANES" BY HUGH O. HOUSE AND LESLIE N. GAY, AND "NASAL IONIZATION—PRELIMINARY OBSERVATIONS" BY H. L. ALEXANDER.

DR. HARRY S. BERNTON.—I should like to ask Dr. House whether he has made any further study of the mucous membrane of his cases.

DR. HARRY P. SCHENCK.—Our results with zinc ionization or with any other therapeutic measures have been comparable to those of Dr. House and Dr. Gay. The main advantage of this method appears to me to be the profit made by the instrument manufacturer. The machines originally sold for from $300 to $400. As you are employing galvanic current, whether you are using a $400 machine or one which can be well constructed for some $30 makes little difference.

This method of influencing allergy has been known to otolaryngologists for a long time. They did not employ zinc ionization, but they have employed trichloracetic acid and chromic acid, and in recent years alcoholic injections. Palmer of New York has employed the application of phenol to the mucous membrane, and his results are comparable to the results obtained by this method. I find in reading the reports of men who have used ionization, that they always fail to include cases which have been treated by these other methods. Until they run a comparable series in which these other substances are used, I do not think that we can claim any advantage for this method.

There is one disadvantage in all of these procedures, including this one. There is a change in the tissue. Recently, Hansel, McMahon, Proetz, Goodyear, and others have shown histologic studies of tissue taken from patients or from dogs in which ionization has been employed, and they find that in those mucous membranes usually taken from the turbinates and the septum there is thickening of the submucosal tissue and an enormous amount of fibrosis. We do not know what that is going to do in ten years from now. Suppose atrophic rhinitis results; there is no cure for atrophic rhinitis, so that the end stage may be worse than the first.

DR. F. K. HANSEL.—Last summer I subjected about 15 patients with nasal allergy and hay fever to the ionization treatment, mostly with the idea of investigating the pathologic changes in the mucosa. Shortly after the ionization, the nose fills with a serofibrinous exudate which remains for about four or five days. The histologic studies of these exudates showed a marked infiltration with neutrophiles and a few eosinophiles. Some of the exudates also showed strips of epithelium with some of the subepithelial tissue attached to them.

Lantern slides, loaned to me through the courtesy of Dr. B. J. McMahon of St. Louis, show some of the changes in the tissues obtained by experimental ionization of the nose of dogs. The first slide represents a section of normal mucous membrane of the dog showing the structure of the epithelium and the subepithelial stroma. The second slide represents the changes in the tissues eighteen hours after ionization. There are changes in the epithelium and a marked acute inflammatory reaction with distended blood vessels and some hemorrhage and edema in the subepithelial tissues. The third specimen removed two weeks after the ionization shows some fragmentation of the epithelium and some areas of regeneration. There is a marked inflammatory reaction of the subepithelial tissues. The fourth slide represents a section taken seven weeks after ionization. It shows a marked increase in the connective tissue throughout the subepithelial layer.

In several patients who had small nasal polyps, specimens were removed two weeks after ionization for histopathologic study. These specimens showed evidence of necrosis. In one particular specimen

the polyp showed necrosis only on one side, this representing apparently only that area which was subjected to ionization.

Although this group of 15 patients is too small from which to draw any conclusions regarding the results obtained from ionization, there were two patients, however, who developed an acute maxillary sinusitis from this procedure. In one of the patients it was necessary to perform an antrum operation to clear up the infection. The plugging of the nose for four or five days with a serofibrinous exudate is somewhat comparable to the insertion of a gauze pack, as is sometimes necessary following a nasal hemorrhage. Among rhinologists it is generally known that prolonged packing the nose in this manner may result in a complicating infection of the sinuses.

DR. M. A. RAMIREZ.—Last year we ran a series of 100 cases of seasonal pollinosis and 25 cases of perennial rhinitis the cause of which could not be demonstrated. We treated each patient by zinc ionization. The results in all the 100 patients were unfavorable. The patients were divided into various groups. Some were treated several weeks before the expectant date of pollination, some immediately before, and some during the season. The results were uniformly bad. In the nonallergic or in the patients in whom we could not find the cause, I do think that there was some benefit derived. We hesitated to report this group because we were told by the men who have reported good results that undoubtedly our technic was faulty, so possibly that is so.

We believe, however, that combining the ionization with injections of pollen probably has some benefit. It does lessen the local sensitivity of the membranes. I do not believe it should just be set aside because of the poor results we have obtained so far. We have been using two methods, one applying 10 milliamperes for ten minutes and the other 5 milliamperes for twenty minutes. In the case in which one uses 5 milliamperes for twenty minutes, the reaction is really very slight; the patients do not complain and the treatments can be repeated.

Giving the treatments during the season seems to give better results than if they are given before the season. Up to the present time, however, our results have been uniformly bad.

DR. PARLATO.—This problem of ionization, like the old adage, proves there is nothing new in history. Over a century ago ionization was first used medically.

As one who has also been doing nose and throat work along with allergy, I have been impressed considerably by the claims for ionization, but there are certain effects which we must always keep in mind when we apply any agent to the nose, especially where that agent causes any trauma to the mucous membrane.

In looking over the literature, I have been impressed, first, by the fact that there has been no mention made of what happens to the eye symptoms in these cases of hay fever and vasomotor rhinitis treated by ionization. No mention has been made of what happens to the cough that occurs in these cases of pollinosis. As an explanation of why some of these patients with vasomotor rhinitis do improve under ionization, I am inclined to offer this hypothesis: We all know that the absorption of protein, autogenous or nonspecific protein therapy

in the form of injection always, or almost always, will produce some kind of alleviation in allergic symptoms. It is quite possible that this coagulation necrosis which occurs in the nasal mucosa, the absorption of that protein can relieve symptoms. While one should not close one's mind entirely to this method of therapy, on the other hand, one should not be too enthusiastic because it is quite possible that rhinologists may go too far just as allergists may not go far enough in the consideration of this subject.

There are a few points that have occurred to me which must be explained before we can accept ionization. These are the sequelae of any such procedure in the nose, whether ionization or cauterization. We have, as Dr. Schenck referred to, atrophic rhinitis, one of the most chronic and difficult conditions to treat. The deposition of connective tissue and scar tissue in the nasal mucosa and subepithelial layer may lead to the destruction of these important tissues of the turbinates. Then I want to call your attention to the synechiae which will form as a result of such traumatizing of the nose.

Last fall, I had two patients suffering from pollinosis who developed asthma for the first time following ionization. The accompanying trauma to the nose may have brought on the asthma.

Dr. Abraham Trasoff.—A few patients with bronchial asthma and atopic rhinitis on whom this method was tried gave the same results described by Dr. Schenck and Dr. House. I merely wish to stress Dr. Parlato's remarks. Dr. Alexander's report that ionization on one side of the nose resulted in improvement on the other side would tend to prove that it is a protein reaction rather than due to the local tissue destruction. I should like to suggest to the men who use ionization to divide their patients into two groups, and to use some foreign protein, such as B. typhosus, intravenously, and ionization in the other group. Such a test, if properly done, would, I believe, settle this moot question.

Dr. Fernand Vistreich.—I had occasion to follow up quite a large number of locally treated hyperesthetic rhinitis patients. Among them I took particular interest in those patients in whom the treatment did not bring any success; and my observations, summarizing them very briefly, lead me to the following statement:

These locally treated cases are usually considered from a very one-sided point of view. The treatment in question, whether it is ionization or chemical cauterization, is given and other factors are not taken into consideration. In cases of failure I found that besides the cauterization, these patients required medication from various angles. They had marked local nasal pathologic conditions, or they were markedly allergic, or there was some endocrine disturbance present, or there were some other reasons which were less serious.

In a series of 20 patients I was able to amplify the beneficial effect of the local cautery by correcting the local nasal pathologic condition or by establishing the various allergic sensitivenesses of those individuals and giving them proper instructions as to the elimination of the antigens or desensitizing them to a certain extent. I would advocate a comprehensive principle in studying the locally treated hyperesthetic rhinitis cases; they should be considered from a broader point of view.

DR. GAY.—We have had enough discussion, and therefore I will be brief. It is our opinion that ionization of the mucous membranes fails to relieve patients suffering with pollen hay fever. The severe reactions with which a number of individuals have suffered following ionization contraindicate its use as a method of treatment in specifically sensitive individuals. To patients who suffer from a local nonallergic rhinitis, it may be of some aid in giving relief. But this problem does not concern internists; it should be dealt with by trained laryngorhinologists.

DR. ALEXANDER.—I entirely agree with what Dr. Gay has said, that in a specifically sensitive individual we do not feel this method of treatment is justifiable. It should not, however, be condemned entirely since it has proved of much value in that small group of patients with vasomotor rhinitis, with negative skin reactions, in whom no etiologic factor can be established by exclusion methods. Under these conditions the first requirement is that one side be ionized. This is really important because with both sides ionized, there is often great discomfort. With but one side done there is far less pain and headache, and occasionally the other side may clear up without further treatment.

Department of Reviews and Abstracts

Announcement

Beginning with the January, 1936, issue of the JOURNAL, the section Selected Abstracts will be much enlarged in order to cover a constantly increasing bibliography on the various aspects of allergy. There will be a division into several sections. Each of these, devoted to one of the specialized fields or to the more important clinical manifestations as asthma and hay fever, will be under the direction of an authority selected by the editorial board.

The director of each section will select the important articles in the current literature. In addition, he will contribute an annual review wherein the year's production will be not only assembled but also interpreted. There will be six such sections besides a general section, so that one annual review will appear in each of the six issues of the JOURNAL.

The editorial board feels fortunate in having secured the following men to serve in this capacity, and acknowledgment is herewith made of their cooperation:

Asthma—Dr. Samuel Feinberg, Chicago
Dermatology—Dr. Marion Sulzberger, New York
Hay Fever—Dr. Will Spain, New York
Immunology—Dr. Matthew Walzer, Brooklyn
Otolaryngology—Dr. French Hansel, St. Louis
Pediatrics—Dr. Scott Smyth, San Francisco

General abstracts will include articles on gastroenterology, migraine, and other subjects related to allergy not considered above.

—The Editors.

Selected Abstracts

Arteriography During Anaphylactic Shock in a Rabbit. Pasteur, Vallery-Radot, Ledoux-Lebard, Hamburger, J. Hugo, A., and Calderon, G.: Presse méd. **43**: 1057, 1935.

In the course of studies on anaphylactic shock in rabbits, the authors have obtained a vasoconstriction in certain areas of the arterial system occurring at the same time as the fall in blood pressure. This, according to the earlier researches of Arthus, is the principal sign of shock. It had been previously shown by the authors that the arteries of the mesentery go into spasm, and also some of the peripheral arteries. In order to obtain more precise information the authors used a Dos

Santos apparatus which permits the injection of a contrast substance into the arteries under a constant pressure. A colloidal suspension of thorium bioxide was introduced into the femoral vein and x-ray pictures were taken.

Nine sensitized rabbits were used which had received three subcutaneous injections of 5 c.c. of horse serum at three-day intervals. The shocking dose was given about three weeks later. Just before this, a manometer was attached to a cannula in a carotid artery, and blood pressure readings were taken. Then thorium was introduced into the femoral artery and an x-ray plate taken immediately. Then 2 c.c. of horse serum were injected intravenously into an ear vein. As soon as a fall in arterial pressure denoting shock was recorded, thorium (10 c.c.) was again injected through the femoral cannula and another picture taken.

In 8 of the 9 rabbits so studied a very marked vasoconstriction of the femoral artery and its branches was readily observed at the time of a lowered systemic arterial pressure.

Temporary Effacement of Sensitization Rather Than Desensitization as a Result of Habituation: Interpretation of the Mechanism of the Action of Method of Prevention of Simple and Anaphylactic Shocks. Arloing, F., and Langeron, L.: Presse méd. **43**: 1, 1935.

For some years the authors have held that in animals or in man sensitive to foreign proteins, the introduction of the specific antigen does not do away with sensitization (does not desensitize), but the subject apparently loses his sensitivity because he has gradually built up a tolerance to the injected protein. Fundamentally, however, his tissues are still sensitive. This conception is enlarged by an exposition of the fundamental principles of anaphylaxis. It is emphasized that such a viewpoint is important because sensitization may be overcome by other means than the introduction of the specific antigen. Nonspecific therapy by injection of foreign proteins and other substances will likewise accustom the organism to the allergens to which it is sensitive. Examples of this phenomenon are given, and the value of nonspecific therapy properly given is stressed.

Biologic Allergy in Newborn Infants. Mayerhoffer, E.: Wien. klin. Wchnschr. **85**: 57, 1935.

Mayerhoffer analyzes at length biologic allergic tissue reactions in a broad sense and then sets out to demonstrate that many symptom-complexes observed in newborn infants, each designated by the name of a given disease, are all, in reality, biologic manifestations of allergy. The various disorders are due to a sensitization acquired through placental tissue. The following conditions are considered

allergic, and in each instance the reason for this opinion is stated at length: toxic erythema of the newborn, pylorospasm, melena neonatorum, laryngeal edema with Charcot-Leyden crystals in the exudate, and dyspepsias. The present studies are a continuation of a program on the subject much of which has previously been published.

Allergic Reactions to Actinomycetes. Mathieson, D. R.; Harrison, R.; Hammond, C.; and Henrici, A. T.: Am. J. Hygiene **21**: 405, 1935.

Infection with actinomycetes tends to produce sensitization in experimental animals, and continued immunization leads to desensitization. In man, normal individuals show more marked skin tests to *Actinomyces bovis* than do patients with actinomycosis.

The Treatment of Milk Allergy and Its Basic Principles. Ratner, Bret: J. A. M. A. **105**: 934, 1935.

The sensitivity to milk, which occurs in adults as well as in children, is acquired either before birth or during early infancy, in the opinion of this author.

Of the different proteins found in milk the casein of cow's milk is similar to that of other species including the human. The whey proteins, lactalbumin and lactoglobulin, on the other hand, are different for each species, and it is these proteins that are responsible for shock reactions in sensitized persons, according to experiments the author has conducted using purified proteins.

To produce shock, protein must be soluble and in its native state. Whey proteins are unchanged by the digestive processes and are easily absorbed into the blood through the intestinal wall. When taken by a pregnant woman they pass through the placenta into the fetus.

Tests with various forms of processed milks have shown evaporated milk to be the best tolerated in cases of milk sensitivity. The long heating process causes coagulation of the whey proteins, reducing their power to produce allergic reactions by making them less soluble. Drying or acidifying milk does not seem to change the whey proteins sufficiently for these forms of milk to be of equal value.

Treatment is based on building up tolerance to milk. Evaporated milk is the only form that can be tolerated, but even this should be boiled vigorously for several minutes before use in severe cases of allergy. All milk-containing foods should be eliminated. Raw milk should be given at the rate of one drop or less daily, gradually increasing the quantity until after from six to nine months the patient should be able to take a normal amount of raw milk. In moderate amounts, raw milk should be given continuously throughout life, or immunity will be lost.

The prevention of milk allergy depends upon: (1) use of moderate amounts of raw milk during pregnancy, especially during the last few months; (2) no isolated raw milk feedings for the newborn or breast-fed infants; (3) no raw milk given during convalescence from severe illness or during or after gastrointestinal disturbances; (4) no excessive or exclusive raw milk diets; (5) avoidance of milk injections for nonspecific therapy.

Allergy and Cataracts. Daniel, R. K.: J. A. M. A. **105**: 481, 1935.

A rather scant literature of the association of cataract with skin disorders, presumably allergic, is reviewed. The author reports three cases, two girls each aged seventeen years and a man of thirty-five years. A definite family history of allergy is mentioned in one. All had an associated fairly severe eczema; and, according to the history, the beginning of cataract formation was definitely associated with the persistence or exacerbations of the skin lesions. The author concludes that the term "allergic cataracts" or at least "cataracts associated with allergy" appears justified.

Cold Urticaria Following Measles in Three Sisters. Kobacker, J. L., and Parkhurst, H. J.: J. A. M. A. **105**: 662, 1935.

In June, 1934, three sisters aged eight, eleven, and thirteen years had measles. No medicine was given. Immediately following recovery all three developed hives in areas of the skin exposed to cold, such as on pressure against a cold milk bottle. Cubes of ice placed on the backs of the hands induced wheals after a few minutes. There was no history of allergy. The only significant laboratory finding was an eosinophilia in each case (7, 10, and 13 per cent). The phenomenon subsided after several months and disappeared entirely a year later.

Status of Allergy Teaching as Indicated in Medical School Announcements. Tumpeer, I. H: J. A. M. A. **105**: 744, 1935.

This summary was made from catalogues of 79 medical schools. In 60 of these allergy was not mentioned. In the remaining 19, teaching of the subject was conducted in most instances in the department of medicine, usually in the fourth year. The courses were usually elective, and the number of hours devoted to them varied from 2 to 16. From the review it appears that allergy is inadequately taught or taught not at all in the majority of medical schools, according to data secured from catalogues. It is conceded that more attention is probably paid to allergy than is indicated in these publications.

The Use of Maize Oil (Unsaturated Fatty Acids) in the Treatment of Eczema: Preliminary Report. Cornbleet, T., and Pace, E. R.: Arch. Dermat. & Syph. **31**: 224, 1935.

Eczema in this study is defined as a condition referred to as "allergic eczema" or "exudative eczema" or "generalized neurodermatitis." In most cases the condition began in infancy, with exacerbations at intervals. The skin was usually thickened and scaly. Eighty-seven such cases, all more than five years of age, and most of them adolescent boys and girls and young adults, were treated. These patients did not recover readily on usual therapy.

Maize oil was given in tablespoonful doses, either before or after meals, whichever was tolerated best. It was increased until four tablespoonfuls were taken three times a day. A specially refined oil with less disagreeable taste was used.

The average time necessary to achieve clinical cure was from twelve to eighteen months. Associated asthma in a few patients was likewise benefited. The improvement seems permanent, and there have been but few relapses since the treatment was begun four and one-half years ago. Some of these patients remained well as long as three years.

The Asthma Research Council: Report of Progress for Year Ended Oct. 31, 1934. Heaton, T. G.: Canad. M. A. J. **33**: 313, 1935.

Among the reported findings, many of which confirm previous experience, are the following statements:

Experiments have proved the superiority of the intradermal skin test over the scratch technic. Positive skin reactions occur in normal people. What is pathologic is the persistence of hypersensitiveness to an abnormal degree.

The general tendency of asthmatic persons who seek medical advice is to improve, whatever the treatment prescribed.

At St. Mary's Hospital a sufficiently large dosage of pollen to abolish the skin test is aimed at, and this necessitates a large number of inoculations. Certain patients have been given treatment sets for self-administration with satisfactory results.

Experiments by Dr. Harley suggest that the state of sensitiveness to bacteria is not induced by the immunizing antigen but by some other bacterial factor.

At Leeds General Infirmary intramuscular injections of liver extract at frequent intervals have been found helpful in the treatment of asthma.

At King's College experiments on the action of adrenalin have shown it to cause a large increase in the blood potassium in animals. Potassium salts, however, have been found to be of no clinical value.

Skin Reaction to Gold and the Gold Treatment for Pulmonary Tuberculosis. Chiucini, G., and Aradas, A.: Policlinico (sez. prat.) **42**: 365, 1935.

Definite results were obtained by skin reactions to gold salts when these were injected intracutaneously in 1 mg. doses in tuberculous patients. Of 20 women tested, there were 12 more or less positive and 8 negative reactions. All patients with positive reactions varying from a small red spot with slight infiltration to strongly positive tests with marked infiltration and redness consistently showed intolerance to gold treatment. All patients with negative reactions took gold without ill effect. The skin reactions to tuberculin and to gold were not parallel.

Intramucous Autoserum Therapy for Bronchial Asthma. Moruzzi, D.: Policlinico (sez. prat.) **42**: 905, 1935.

This work was done to confirm that of Jacquelin and Bonnet, by injecting autoserum into the nasal mucosa. From 0.2 c.c. to 0.5 c.c. to as much as 1 c.c. was given daily. In 11 chronic asthmatic patients with severe paroxysms, 3 were completely relieved, 4 were made distinctly better, and there were no results in the remaining four other than a reduction of eosinophilia. In allergic bronchitis the treatment was likewise found to be applicable.

Atopic Annoyances in the Course of Pulmonary Tuberculosis. Phillips, E. W.: Ann. Int. Med. **8**: 1649, 1935.

Atopic manifestations, especially hay fever, are often seen in cases of pulmonary tuberculosis. The clinical material studied was that usual to sanatoriums in the Southwest. Two hundred and fifty consecutive tuberculosis patients are included in this study. Fifty patients, one-fifth of the total number, showed during the period of observation clinically evident sensitization to identified foreign proteins. Thirty-four of these were pollen sensitive. About half of these had simple hay fever easily controlled by specific therapy. The remaining half presented atypical symptoms of increased cough and increased expectoration. These symptoms were likewise controlled by pollen therapy. Sensitivity to foods alone occurred in about one-third of the atopic patients. The symptoms in these cases resembled the digestive disturbances frequently incident to the toxemia of tuberculosis. These were relieved by exclusion diets, but food should not be restricted to the point of causing continued loss of weight. The recognition and the proper management of sensitivity to foreign proteins constitute a useful addition to the hygienic treatment of tuberculosis.

The Allergic Factor in Colitis. Mogena, H. G.: Arch. d. mal. de l'app. digestif. **25**: 57, 1935.

Fifty cases with mucous or catarrhal colitis were studied from the standpoint of bacterial allergy. The colon bacillus, enterococci, and Bargen's diplococcus were found in 84, 42, and 40 per cent of cases respectively. Sensitivity was tested for intradermally with heat-killed cultures, and reactions were looked for after one-half hour, twenty-four hours, and forty-eight hours. They were considered positive only when they reacted in one-half hour and were negative at later readings. Control tests on normal individuals were done. Sensitivity to colon bacilli occurred in 48 per cent, to enterococci in 24 per cent, and less frequently to other organisms (paracolon bacilli, *B. fecalis*, staphylococci). In every patient, sensitivity was multiple. Vaccine therapy with the implicated bacteria was better than with any other form of treatment. No exacerbations occurred from the use of vaccine.

Eleven cases of ulcerative colitis were likewise studied. Bargen's organism predominated in these. Vaccine therapy was not effective.

The Value of Hyperpyrexia in the Treatment of Bronchial Asthma. Phillips, K., and Shikarry, S.: Southern M. J. **28**: 801, 1935.

Fifty patients with bronchial asthma, regardless of sex, age, type of asthma (acute, chronic, intractable), previous treatment, or other associated factors, were selected for study. Each patient was treated with one course of hyperpyrexia consisting of ten treatments. Patients were kept under observation from three to eight months. Eighty-two per cent were relieved, but after six months only 32 per cent remained so. Various laboratory data were secured on the patients studied. No prolonged alteration of the blood count, blood sugar, nonprotein nitrogen, uric acid, acid-base balance, or blood calcium occurred. The only lasting change was a rise in metabolic rate which was definitely increased following a course of treatment.

The Treatment of Asthma With Especial Reference to the Oral Use of Adrenal Hormones and Sodium Chloride. Pottenger, F. M., and Pottenger, R. T.: California & Western Med. **43**: 10, 1935.

On the basis that the vegetative nervous system is probably in imbalance in asthmatic persons, adrenal extracts, both as epinephrine and as adrenal cortex, were administered by mouth. In addition, large doses of sodium chloride (3 to 6 gm.) were given in 240 c.c. of water one-half hour before meals. This sodium salt is known to enhance adrenal activity. Alone it did not control asthma, but combined with extract the results were effective. Of 50 patients so treated, 28, including 19 children and 9 adults, were considerably relieved of

their asthma. When recurrences did occur after infections and other causes, they again responded to treatment. Of the cases reported, only two patients received no benefit.

The Control of Bronchial Asthma. Shambaugh, N. F., and Alter, S. M.: Science **81**: 210, 1935.

The principle of treatment is postural drainage. The patient kneels on a chair or stool and places both hands on the floor in order to have the thorax assume as nearly a vertical position as possible. The patient then coughs as nearly continuously as possible, which permits a discharge of exudate. The position is maintained for at least three minutes regardless of the amount of exudate produced. The procedure is carried out twice a day presumably on arising and on retiring. Cough during the interval indicates that insufficient drainage has been accomplished. This procedure is used for the prevention of paroxysms; although not until underlying etiologic factors, such as infectious foci, are eradicated will permanent relief be secured.

The authors' cases consist of patients between the ages of six and seventy-five years, and all have been markedly benefited. Reduced viscosity of the sputum is necessary for its discharge.

Arrest of Asthmatic Crisis by Means of Urticaria Provoked by Scarifications With Histadin. Israel, L., and Jacob, A.: Soc. de Therap. June 12, 1935. (Abstract in Presse med. p. 1288, Aug. 14, 1935.)

The authors stopped an asthmatic crisis with the following technic: With a sterile knife a series of scarifications, lengthwise and horizontal, were placed about 1 cm. apart on the internal aspect of the arm or forearm or on several other parts of the skin. On these they placed drops of a solution of histadin hydrochloride, 4 parts in 100. The urticarial wheals soon disappeared.

Erratum

In the September issue of the JOURNAL in the article "Treatment of Allergic Patients With Added Skin Reactions" by Joseph B. Biederman, in the second paragraph on page 500 the last sentence should read: . . . "one patient in 254 developed symptoms to an additional pollen to which he was skin *negative* at a different season, the following year."